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A COMPARATIVE STUDY OF THE CHROMOSOMES IN DECAPODS, ISOPODS AND AMPHIPODS, WITH SOME REMARKS ON CYTOTAXONOMY AND SEX-DETERMINATION IN THE CRUSTACEA

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I. INTRODUCTION

The present investigation was undertaken with a hope to discuss the bearing of cytology upon the mechanism and processes of evolution, and to make a new approach to classical problems of morphological taxonomy in certain groups of the Crustacea. At present, it is generally accepted that, so far as the higher animals are concerned, all evolutionary transformations have had their origin in the chromosomes, and that they furnish the material source of evolutionary changes, since they constitute the physical basis of heredity (White '57a). Therefore, any significant alteration in the structure and behaviour of the chromosomes represents an evolutionary change. The differences in chromosome numbers, and chromosome patterns which frequently distinguish one species from its relatives throw new light on the problem of taxonomy. These considerations will reasonably also be applicable for lower animals such as Crustacea.

The problem how and why related species of organisms have acquired visibly different chromosome sets, or karyotypes, in the course of their evolution, has long attracted the prime interest of cytologists. The progress of genetics has led to the understanding that each of the characters of an organism represents the results of the interaction of genes (White '54). It should be stated therefore that the nature of organisms will be more exactly mastered from the viewpoint of cytological knowledge involving chromosome constitution than from the morphological standpoint. Cytologists are concerned with the cytotaxonomic differences which exist between related species. Such differences may sometimes be used to distinguish sibling or cryptic species that cannot be separated on the basis of merely external characters. They are the results of chromosomal rearrangements which have arisen spontaneously and established themselves in the course of phylogeny. The cytogenetic data thus far presented have enhanced the development of cytotaxonomy which is based upon cytological and genetical criteria (White '57b).

As early as 1885, the cytological study of Crustacea was initiated by Carnoy. He reported the chromosomes of four species of Crustacea. Probably due to poor fixation, however, he could not determine the precise number of chromosomes in those species. Following Carnoy's study, a considerable number of papers have been published on the chromosomes of various species of Crustacea. The report pertaining to animal chromosomes published by Makino ('56) indicates that the morphology of chromosomes of about 150 species covering seven orders of Crustacea have been reported by a number of investigators.

In spite of the considerable amount of work done on the chromosomes of Crustacea, knowledge on those chromosomes has remained very limited, remarkably less progress having so far been made in this group of animals than in other groups of Arthropoda, *e. g.* Insecta. Reference to the literature shows that the majority of earlier works with respect to the chromosome morphology of Crustacea, have reported very meagre results, due probably

to the technical difficulties. It is evident that basic data for understanding the evolution of the hereditary mechanism in Crustacea are incomplete. Thus, the need for more exact knowledge of the chromosome morphology in various groups of Crustacea is increasing for the sake of the establishment of the scientific taxonomy of this group of animals. In order to make some contributions to this object, the present author has undertaken a comparative study of the chromosomes in three orders of Crustacea: Decapoda, Isopoda and Amphipoda, with particular regard to the karyological relationship to systematics, under the suggestion and guidance of Dr. Sajiro Makino.

The present paper will describe in the following pages the results obtained in this chromosome survey, with discussion on the deta presented by earlier investigators and with particular concern to the interrelationships of taxonomy.

The present study is divided into three parts. Part I is devoted to the investigation of the morphology of chromosomes in 33 species covering three orders of Crustacea. Beginning with the work of Carnoy on the chromosomes of Crustacea, which appeared as early as 1885, a considerable number of works have successively been published in various species covering several orders of Crustacea by many investigators. The majority of earlier works are not satisfactory from present-day-standards of cytology. To obtain good preservation of chromosomes of Crustacea is a task very difficult, because of the fact that they are marine dwellers. The preservation of chromosomes of marine animals is a matter of difficulty in general. Further, many crustacean species have a comparatively large number of chromosomes which are generally small in size. To advance knowledge of crustacean chromosome morphology a considerable time has necessarily been devoted in the present study due mainly to technical difficulty, to discover the exact morphology of chromosomes.

In Part II will be described the relationship between chromosome morphology and taxonomy of the Crustacea. Chromosome numbers vary considerably among the different orders. Further, the chromosome numbers vary notably among the subdivisions of the order. Attention in this study was directed to the problem of whether any relation exists between chromosome number and taxonomical classification considering the evolution of chromosome complexes,.

Part III deals with the sex-determining mechanism in Crustacea in relation to the chromosomes. Following the pioneer work of Braun ('09) and Matschek ('09, '10) on the sex-chromosomes of Copepoda, many reports have been issued on the sex-mechanism of Crustacea; on Phyllopoda by Baker & Rosof '27, '28; on Ostracoda by Bauer '34, '40 and Dietz '54, '58; on Copepoda by Braun '09, Matschek '09 '10, Heberer '32 and Beervann '54; on Isopoda by Dworak '35 and Steiger & Bocquet '54; on Decapoda by Fasten '14, Leopoldseder '34 and Delpino '34; and on Amphipoda by Palmer '25, '26. The data presented by these authors seem to contain some doubts in more or less degree as considered from present-day-standards. Attention in this study was directed to the question whether sexual difference of chromosomes would really exist and, if they do exist, what type of

sex-mechanism of the Crustacea would occur in relation to the other groups of animals.

Before going further, the anthor's hearty thanks are due to Emeritus Professor Kan Oguma for his valuable advices. Also, the author wishes to acknowledge here his very great indebtness to Dr. Sajiro Makino, Professor of Zoology, Faculty of Science, Hokkaido University, who has offered continuous guidance and encouragement, for his kindness in revising and improving this manuscript. Further the author's thanks should be extended to Dr. Saburo Saito, Professor of Zoology, Faculty of Fisheries, Hokkaido University, who has given strong support in various ways to the completion of the present investigation.

II. MATERIAL

The following 33 species of crustaceans provided the material for the present study. They are classified into three Orders:

ORDER DECAPODA

Suborder Natantia	
Group Penaeidea	
Family Penaeidae	*Penaeus japonicus* (BATE)
Suborder Reptantia	
Group Palinura	
Family Palinuridae	*Panulirus japonicus* (V. SIEBOLD)
Group Astacura	
Family Potamobiidae	*Cambarus clarkii* (GIRARD)
	Cambaroides japonicus (DE HAAN)
Family Nephropsidae	*Nephrops japonicus* TAPPARONE-CANEFRI
	Nephropsis carpenteri WOOD-MASON
Group Anomura	
Tribe Galateidea	
Family Galateidae	*Cervimunida princeps* BENEDICT
Tribe Paguridea	
Family Paguridae	*Eupagurus ochotensis* (BRANDT)
Family Coenobitidae	*Coenobita rugosa* H. MILNE-EDWARDS
Family Lithodidae	*Paralithodes camtschatica* (TILESIUS)
	Paralithodes platypus BRANDT
Group Brachyura	
Tribe Oxystomata	
Family Carapidae	*Matuta lunaris* (FORSKÅL)
Family Leucosiidae	*Philyra pisum* DE HAAN
Family Raninidae	*Ranina ranina* (LINNÉ)
Tribe Brachygnata	
Superfamily Oxyrhyncha	
Family Majidae	
Subfamily Inachinae	*Machrocheira kaempferi* DE HAAN
Superfamily Brachyrhyncha	
Family Portunidae	
Subfamily Portuninae	*Ovalipes punctatus* (DE HAAN)
Subfamily Lupinae	*Scylla serrata* (FORSKÅL)
Family Atelecyclidae	*Telmessus cheiragonus* (TILESIUS)
Family Xanthidae	*Atergatis floridus* LINNÉ
Family Grapsidae	
Subfamily Grapsinae	*Pachygrapsus crassipes* RANDALL
Subfamily Varuninae	*Hemigrapsus sanguineus* (DE HAAN)
	Hemigrapsus penicillatus (DE HAAN)
	Eriocheir japonicus DE HAAN
	Gaetice depressus (DE HAAN)
Subfamily Sesarminae	*Sesarma intermedia* (DE HAAN)
Subfamily Plagusiinae	*Plagusia dentipes* DE HAAN

ORDER ISOPODA

Suborder Oniscoidea	
Family Ligiidae	*Megaligia exotica* (ROUX)
Family Tyliidae	*Tylos granulata* MIERS
Suborder Valvifera	
Family Idoteidae	*Cleantiella isops* (GRUBE)
	Idotea japonica RICHARDSON
Suborder Flabellifera	
Family Sphaeromidae	
Subfamily Sphaerominae	*Cymodoce japonica* RICHARDSON
	Tecticeps japonicus IWASA

ORDER AMPHIPODA

Suborder Gammaridea	
Family Gammaridae	*Anisogammarus annandalei* (TATTERSALL)

III. TECHNIQUE

For the study of chromosomes, the testes which were removed from animals in living state immediately after capture were exclusively adopted as material. After several trials in fixation, the present author was successful in finding a new fixative which is a modified formula of weak Flemming's mixture for the purpose of such a study as the present investigation. This fixative proved the most excellent so far tried for preservation of the chromosomes of every species of Crustacea. The contents of this mixture are as follows : –

2% Osmic acid	5 c.c.
1% Chromic acid	25 c.c.
0.1% Acetic acid	10 c.c.
Distilled water	60 c.c.

The testes were allowed to rest in the above fixing fluid for 20 to 24 hours ; the material was washed thoroughly in running water for the same length of time. After being dehydrated with ascending grades of alcohol in the usual way, the material was cleared in creosote-toluol and toluol, then embedded in paraffin. Sections were cut 10 micra in thickness. After bleaching with hydrogen peroxide, the sections were stained by the iron-haematoxylin method after Heidenhain.

All the figures were drawn with Abb's drawing apparatus, using a Zeiss H. I. 90 obj. and a K20× compensating ocular, at the level of the desk on which the microscope was set. The magnification rate of all the figures was 3700 times in diameter.

IV. PART I. COMPARATIVE MORPHOLOGY OF CHROMOSOMES IN THIRTY-THREE SPECIES OF CRUSTACEA

ORDER DECAPODA

1) *Penaeus japonicus* (*BATE*)

The present species is an edible prawn famous in Japan for its delicious taste. It

belongs to to the Family Penaeidae, Penaeidea, Natantia of the Order Decapoda. The prawns from which the material of the present study was obtained, were captured in shallow water at Kisarazu, Chiba Prefecture by fishermen in May, 1943.

Though the dividing figures of spermatogonia were scanty among the testicular cells, a few good metaphase equatorial plates could be observed. Fig. 1 is an illustration of them. In the polar view, the chromosomes appear taking a usual radial arrangement forming a circular equatorial plate. After careful analysis of chromosomes, it was found that the diploid complex consisted of 14 V-shaped metacentric elements and 78 acrocentric ones showing a gradual diminution of size from rod to dot. The metacentric chromosomes are arranged into 7 homologous pairs, according to their sizes and shapes. They all have nearly submedian attachment of the spindle fibres. It is very difficult to determine the homologous pairs among the acrocentric chromosomes, except for several larger ones. The diploid number of this species, therefore, was ascertained to be 92 which are formulated as 14V'+78R' in the male.

Division takes place synchronously in all spermatocytes within a cyst. The dividing figures of spermatocytes, therefore, could be observed in large number. Figs. 2 and 3 are the polar views of primary spermatocyte at metaphase. The number of bivalents in an equatorial plate is 46 without exception. Several greater bivalents assume criss-cross or triangular shape considered as a modified form of the former tetrad. Remaining smaller bivalents take the form of a dumbbell or rectangle . At the side view of anaphase, all the bivalents separate synchronously into two equal halves. There is no chromosome having a character in form and behaviour usual to the sex-determining chromosome.

Fig. 4 is a polar view of a secondary spermatocyte at metaphase. There are 46 chromosomes of dyad nature. Seven of them are V-shaped elements and the remaining ones are rod- and dot-shape. These 7 V-shaped dyads are evidently the descendants of V-shaped chromosomes in the spermatogonial garniture. At anaphase they divide synchronously into equal monads. Also at this division no particular chromosome in either form and behaviour could be observed.

The chromosome number of the present species is 92 (14V'+78R') in diploid and 46 in haploid.

*2) **Panulirus japonicus** (**V. SIEBOLD**)*

This species is well known in our country as a tasty edible spiny lobster of the Family Palinuridae, Palinura, Reptantia of the Order Decapoda. The lobsters from which the material of the present work was obtained were collected by nets in waters adjacent to the Mitsui Institute of Marine Biology at Suzaki, near Shimoda, Izu Peninsula, at intervals from March to August in the years, 1933 and 1935.

In the testis, the cells contained in a cyst are of the same phase ; some cysts are filled with only spermatogonia, while others are provided with spermatocytes of different kinds, primary or secondary.

The spermatogonial chromosomes assume a rosette form in their metaphase arrange-

ment, as they lie radially in the equatorial plate. They are found well apart from one another with sharply defined contour, so as to make the counting of chromosome number very easy in spite of their large number. In order to determine the number and form of the chromosomes, many equatorial plates were examined from their polar aspect. Examples are given in Figs. 5 to 6, every one of which overlapping of chromosome occurs to a slight degree. After careful counting, it was decided that the diploid complex consists of 140 chromosomes. As is recognizable in the figures, the spermatogonial garniture is of polymorphic nature ; of 140 chromosomes, 12 are V-shaped metacentric elements while the remaining ones are all acrocentric rods showing gradual diminution of size. According to the size and shape the metacentric chromosomes are arranged into 6 homologous pairs, of which three pairs are rather large as compared with the remaining three. They all have nearly submedian attachment of the spindle fibres. Among the acrocentric chromosomes, it seems impossible to know which two's constitute the homologous pairs, except for some larger ones.

The chromosomes of the primary spermatocytes are observed with absolute clearness in the polar view of the metaphase plate, as they scatter evenly and are distinctly separated from each other. As the usual case may be, the larger ones take the peripheral position with those of smaller size lie in the central region of the spindle. As expected from the count of the spermatogonial chromosomes, a primary spermatocyte contains invariably 70 bivalent chromosomes (Figs. 7–8). Of the large sized bivalents found in the peripheral part of the spindle, three are sharply distinguishable from the others in having a striking characteristic in their morphology. They belong to the compound ring-tetrads, composed of two or three rings connected in a series with metacentric fibre attachment. It is certain that these bivalents are the descendants of three pairs of larger V-shaped chromosomes in the spermatogonium. Three more bivalents with metacentric fibre attachment, but small in size, are always found in addition to those just mentioned. They are evidently descendants of three pairs of smaller metacentric chromosomes in the spermatogonium, but whether they have ring structure is not known at present. When viewed from the pole of division, the remaining bivalents exhibt, at least in larger ones, a dumbbell shape being different in size, with a remarkable transverse suture in each middle region. In the smaller bivalents, however, the actual presence of the middle suture is not proven for certain. In the anaphase of the first division, the separation of chromosomes always takes place synchronously.

As naturally expected from the mode of separation of chromosomes in the first division, only one kind of secondary spermatocyte is produced in respect to the chromosome complex. There are also counted 70 dyads without exception in the metaphase equatorial plate of the secondary spermatocytes (Fig. 9). The chromosomes are quite similar in arrangment to those of the spermatogonia and primary spermatocytes, as larger ones are arranged at the periphery of the spindle while dot-like ones are in the central region. Further, all chromosomes corresponding to those of the spermatogonium are to be pointed out too in the

garniture of the secondary spermatocytes : 6 V-shaped metacentric chromosomes and the remaining acrocentric ones from rod to dot shape.

The chromosome number of the present species is 140 in diploid and 70 in haploid in the male. The polymorphic nature of the chromosome complex consisting of the formula 12V'+128R', is a remarkable character of the species. Such a condition of chromosomes is very interesting from the morphological viewpoint, when one considers the evolution of the chromosome complex in Crustacea. There is no chromosome to be considered as the sex-chromosome in form and behaviour.

3) *Cambarus clarkii* (*GIRARD*)

The present species is a crayfish originally introduced from America in 1930 and propagate abundantly throughout Japan in paddy-fields. It belongs to the Family Potamobiidae, Astacura, Reptantia of the Order Decapoda. The materials for the present study were obtained from some specimen gathered from a paddy-field near Ohfuna Railway Station, Kanagawa Prefecture in June, 1936.

In the testis many dividing figures of spermatogonia could be observed. Fig. 10 is an example of metaphase polar views of spermatogonia. As seen in the figuret, the chromosomes distribute homogeneously well apart from one another with sharply defined contour in the equatorial plate. The number and form of chromosomes could easily be observed in spite of their large number. After careful counting, it was decided that the diploid complex consists of 192 chromosomes. As in the case of the previous species, *Panulirus japonicus*, the present species also is polymorphic in diploid garniture; of 192 chromosomes, six are V-shaped metacentric elements, while the remaining ones are all acrocentric rods showing gradual diminution of size. Size differences between metacentric and larger acrocentric chromosomes are not so remarkables as in the case of the previous species, *Panulirus japonicus*. According to their sizes and shapes the metacentric chromosomes are arranged into 3 homologus pairs. They all have nearly submedian attachment of the spindle fibres. Since the acrocentric chromosomes show gradual diminution of size, it seems impossible to know which two's constitute the homologous pairs, except for some of the larger ones.

Dividing figures of spermatocytes could be observed abundantly within a cyst. At the polar view of metaphase of a primary spermatocyte chromosomes are observed with absolute clearness, as they distribute well apart from one another forming a round equatorial plate. Fig. 11 is an example of polar view of a primary spermatocyte at metaphase. The number of bivalent chromosomes contained in an equatorial plate is invariably 96. The haploid number of the present species is 96 in the male. When viewed from the pole of division, almost all the bivalents take dumbbell shape form showing a transverse suture in each middle region. Among these bivalents there are a few assuming the shape of a criss-cross or analogous form. Certainly these bivalents are the descendants of three pairs of V-shaped chromosomes in the spermatogonium. Remaining small bivalents are in the form of a rectangle. At anaphase of first division, all the bivalents divide synchronously

into two equal halves and migrate to each pole of the spindle. There is no chromosome particular in form and in behaviour to be considered as the sex-chromosome, nor does any chromosomes group make strange movement as happened to occur in *Cambarus immunis* (?) according to Fasten ('14).

As expected from the mode of the first division, there is only one kind of secondary spermatocyte. Polar view of the metaphase equatorial plate of the secondary spermatocyte is shown in Fig. 12. In the circular equatorial plate there occur 96 chromosomes in dyad nature. Among these, there are three metacentric chromosomes, having submedian fibre attachment and the remaining 93 are acrocentric rod- and dot-shaped chromosomes. At the second division, all the chromosomes divide simultaneously into two equal halves. In this division also no particular chromosomes in form or in behaviour could be detected.

The chromosome number of the present species is 192 in diploid having the formula 6V'+186R', and 96 in haploid in the male. There is neither any chromosome which is in behaviour characteristic to the sex-chromosome, nor any chromosome group taking a strange course at division.

4) *Cambaroides japonicus* (DE HAAN)

The present species is a common crayfish in lakes and streams throughout Hokkaido. It belongs to the Family Potamobiidae, Astacura, Reptantia of the Order Decapoda along with the previous species, *Cambarus clarkii*. The material with which the present study was carried out was mostly obtained in the suburbs of Sapporo, Hokkaido but some were secured from Lakes Tōya and Kussharo, Hokkaido, during June, July and August 1933. The material obtained from the beginning to the end of July proved to be most favourable for the study of chromosomes of various stages.

The spermatogonium is rather large of size and nearly round in shape. Two kinds of spermatogonia, the early and late can be distinguished by their size. The spermatogonium does not undergo simultaneous division in a cyst; the dividing figures are found independently. In the polar view, the chromosomes are noticed to be distributed homogeneously throughout the whole area of the equatorial plate, arranging themselves well apart from one another. It is not very difficult, therefore, to count the number of chromosomes in spite of their large number. Careful counting of many equatorial plates revealed that the spermatogonium contains invariably 196 chromosomes (Figs. 13–14). The chromosomes are mostly short straight rod-shaped, but sometimes slightly curved. They are however, all acrocentric chromosomes. The present species, therefore, has a chromosome garniture of isomorphic nature. It seems to be impossible to identify the homologous pairs except for the longest one. These longest chromosomes always form a pair, being nearly equal in size; they can be easily distinguished from the others.

Fasten ('14) reported 200 dot-like chromosomes to occur in the spermatogonium of *Cambarus virilis*, stating that "the chromosomes are spherical in shape". The present species, therefore, is remarkably different from *Cambarus virilis*, in respect to the shape of

chromosomes, though the two forms do have an isomorphic complex of chromosomes.

In the primary spermatocyte the chromosomes arrange themsevles with extreme clearness in the metaphase plate as shown in Figs. 15 to 16. Therefore, counting of the chromosome number is rather easy in the present stage. In every equatorial plate examined, a garniture of the halpoid chromosomes is, without exception, made up of 98 bivalents constituting a nearly round equatorial plate. The bivalents are generally so disposed in the equatorial plate as to be scattered about throughout the whole space of the latter. Frequently, a small vacant space is found in the central region of the equatorial plate as seen in Figs. 15 and 16, probably due to some mechanical effects in preparation of the sections. When viewed from a pole, almost all the bivalents exhibit a conspicuous transverse suture across their middle region, as a results of which they assume a dumbbell shape. At anaphase, all the bivalents separate symmetrically into two equal halves. The division of the chromosome took place synchronously, neither precession nor succession could be observed in any chromosome. Any particular group of chromsome taking a strange behaviour which was reported by Fasten ('14) to occur in *Cambarus immunis* (?) could not be observed in the present species.

There is only one sort of secondary spermatocyte concerned with the chromosome complex. The chromosomes and the equatorial plates are generally much smaller in size, compared with those of the primary spermatocyte. Careful counting shows, without any doubt, 98 chromosomes of dyad nature in every metaphase plate (Fig. 17). They are all rod-shaped but seem very much shorter than the spermatogonial chromosomes and exhibit dyad nature superimposing the constituent halves with each other. There is nothing particular in the mode of chromosome separation in the present division, every dyad separating equally into two daughter mon ıds. Fig. 18 shows one of the two daughter chromosome garnitures at anaphase in which 98 monads are clearly to be counted. In this group of chromosomes one finds a long chromosome, which is clearly distinguishable from the remaining ones. This chromosome, as may be readily noticed, is that one which corresponds to one of the longest pair found in the spermatogonium. The reason why this large chromosome has never been observed in either primary or secondary spermatocyte divisions, is that the difference in size between the latter mentioned chromosome and the remaining ones is not sufficiently great to distinguish it in a state otherwise than its full extension. The longest chromosome is not considered to be the sex-element, since it divides equally in the maturation divisions and there is nothing unusual in the behaviour.

The chromosome number of the present species in the male is 196 in diploid. They are all of acrocentric rod-shape. The haploid number is 98. Throughout the dividing stages of meiosis, no chromosomes peculiar in heterotropic behaviour characteristic to the sex-chromosome could be observed.

5) *Nephrops japonicus* TAPPARONE-CANEFRI

The present species is a deep-sea edible prawn of the Family Nephropsidae, Astacura, Reptantia of the Order Decapoda. The prawns for the present work were obtained in

April, 1937 from Suruga Bay, to the west of the Izu Peninsula, at depths of 200–400 meters. It is distributed in the deep-sea along the coast of the Pacific of southern Japan from the Bōsō Peninsula to Shikoku.

In the testis, dividing figures of spermatogonia are rather rare. Several excellent figures could be observed among the cells constituting the inner walls of cysts. Fig. 19 is an example of the metaphase polar view of the spermatogonium. As seen in the figure, chromosomes constitute a round equatorial plate distributing themselves well apart from one another with sharply defined contour, so as to make counting of the chromosome number very easy in spite of their large number. The spermatogonial graniture of the present species is isomorphic in nature: all the chromosomes are acrocentric showing gradual diminution from rod to dot in shape. After careful counting, it was decided that the diploid complex consists of 164 chromosomes. There are no chromosomes having particular form. The diploid number of this species, therefore, was determined as 164 in the male.

The division of the primary spermatocyte takes place synchronously within a cyst. The author failed to observe any dividing figures of secondary spermatocytes in the present material. Fig. 20 is a polar view of a primary spermatocyte at metaphase. The bivalents are observed with absolute clearness in the plate, as they scatter evenly and are distinctly separated from each other. About a half of all the bivalents assume the usual type of compact dumbbell shape observed in Decapoda in general. The remaining ones possess a strange quadripartite structure, each consisting of well differentiated quadripartite chromatic elements. The existence of these bivalents will be dealt with in detail in the following species, *Nephropsis carpenteri*. The number of bivalents in the present species in an equatorial plate is 82 without exception. There is present no chromosome taking particular behaviour considered as the sex-chromosome in the primary spermatocyte (Fig. 21). Nevertheless the dividing figure of the secondary spermatocyte could not be observed in the present material. It is supposed from the above results that only one kind of secondary spermatocyte having 82 dyads is produced.

The chromosome number of the present species is 164 in diploid and 82 in haploid in the male. The chromosomes are all acrocentric in nature showing an isomorphic complex. There is not any evidence for the presence of any particular chromosome to be considered as the sex-chromosome, either in respect to behaviour or in structure.

***6) Nephropsis carpenteri* WOOD-MASON**

The present species is also an edible prawn inhabiting the deep-sea. The present form, together with *Nephrops japonicus*, belongs to the Family Nephropsidae. The two prawns are therefore closely related taxonomicaly. The prawns obtained at the same place as the former materials furnished the material for the present study.

Dividing figures of spermatogonia are very rare in the testes of the present material. Two excellent metaphase polar views were fortunately obtained for close study. An example is given in Fig. 22 which shows a minor degree of overlapping of chromosomes. Counting indicates that the diploid complex consists of 152 chromosomes. The

spermatogonial garniture is polymorphic in nature : of 152 chromosomes, 30 are metacentric V-shaped elements, while the remaining 122 are all acrocentric rods showing gradual diminution of size from rod to small dot. It is impossible to identify with confidence the homologous pairs, except for some comparatively large V-shaped chromosomes and some small dot-shaped ones. As a whole, the metacentric V-shaped chromosomes occupy a peripheral position in the equatorial plate surrounding the rod-shaped acrocentric ones in the central part.

The chromosomes of primary spermatocytes are observable clearly in the polar view of the metaphase plate. Since the bivalents are found well apart from one another, counting of their number and observation of their morphology was rather easily carried out. Every equatorial plate shows 76 bivalents with unvarying clearness (Figs. 23–24). Almost all the bivalents with exception of a few smaller ones, exhibit strange quadripartite structure. These bivalents are the same in structure as those observed in the previous species, *Nephrops japonicus*. Observations from a pole show that the quadripartite bivalents are composed of four chromatins attaching two by two. The constituent element of each chromatin mass show uniform size. Each masses of a biualent are connected with two fine threads. Considered from the constitution of the tetrad, this quadripartite structure is the that of the upper half of a tetrads. There must be, therefore, another like quadripartite structure under it attaches closely to it. In other words, a bivalent is composed of eight smaller chromatin elements, of which each four are connected with chromatic fibres. Such a structure is seen in several bivalents among usual quadripartite bivalents. These strange bivalents appear to be haxapartite in structure. It seems to be an opened shape of the above bivalent at one end. When observed at anaphase, the octopartite structure appears in side view of the first division (Fig. 25). Such a structure of bivalents may be due to a loose condensation of chromonema at metaphase of the primary spermatocyte. At anaphase of the first division, no chromosome having special behaviour and to be regarded as the sex-chromosome could be observed.

The chromosome number of the present species in the male is 152 having a formula of 30V'+122R' in diploid and 76 in haploid. On the basis of the fact that almost all the bivalents of the present species and the closely related species, *Nephrops japonicus*, possess a similar quadripartite structure in a half of all the bivalents, it seems probable that the two species under consideration have a close systematic relationship. Further, the chromosome constitution of the closely related two species, *Nephrops japonicus* and *Nephropsis carpenteri*, 164 (all rods) for the former, 152 (30V'+122R') for the latter, is specialy interesting when one considers the evolution of the chromosome complex.

7) *Cervimunida princeps* BENEDICT

The present species is a deep-sea crab, belonging to the Family Galateidae, Galateidea, Anomura, Reptantia of the Order Decapoda. The specimens for the present work were obtained from the same place and at the same time as those of the previous two species, *Nephrops japonicus* and *Nephropsis carpenteri* in April, 1937 from Suruga Bay to the west

of the Izu Peninsula, at depths of 200 to 400 meters.

Dividing figures of spermatogonia were observable among cells constituting the inner walls of testicular cysts. Fig. 26 is an example of well-preserved metaphase plates of spermatogonia. Counting revealed that the diploid complex consists of 18 V-shaped metacentric elements and 91 rod- and dot-shaped acrocentric ones, showing the diploid number of 109 in the male. The odd number, 109 clearly suggests the existence of a sex-chromosome. The acrocentric chromosomes are odd in number and therefore contain any unpaired elements, but they vary in size gradually from rod to dot; the condition makes it impossible to identify any unpaired elements.

The division takes place synchronously in all spermatocytes within a cyst. The dividing figures of spermatocytes, therefore, could be observed in a large number. Every primary spermatocyte under study showed, in side-view, three univalent chromosomes, two being at one side and the other one at the other side of the equatorial plate ; the three lie a considerable distance apart from the remaining chromosomes which arrange themselves in an equatorial plate. The number of bivalents lying on the equatorial plate was found to be 53. Size differences are noted among them in a rather remarkable degree ; some larger ones exhibit the type of ring- and cross-tetrads, while the remaining ones are of dumbbell shape. The larger bivalents may be the descendants of V-shaped chromosomes in the spermatogonium. By changing the focus of the microscope from the equatorial plate up and down, two chromosomes at one side and one chromosome at the other side, or *vise versa,* could be observed in every metaphase plate as shown in Figs. 27 and 28. According to the general rule of the sex-determining mechanism, it is reasonable to assume that these three univalent chromosomes constitute a compound sex-chromosome complex composed of X_1X_2- and Y-elements. The X_1 and X_2 are nearly identical in size, while the Y is slightly larger than either of the two X's (Figs. 31 to 33). The Y is joined separately at one end with each of the two X's. At metaphase, the compound sex-chromosome is always situated considerably off the equatorial plate without exception. It seems therefore that the distance pairing of the sex-trivalent is established by a pairing mechanism during the diakinetic stage, but is not due to the mechanism of precession at metaphase. A comparable case was reported in the male of a neuropteran, *Plethosmylus decoratus* by Hirai ('56). The chromosome formula of the primary spermatocyte of the present species is represented by $53+X_1+X_2+Y$. At anaphase, the two X's and Y separate from each other and migrate to opposite poles together with daughter halves of ordinary chromosomes. The number of chromosomes in the daughter complexes after division is 54 and 55, respectively (Fig. 34, a-b).

The secondary spermatocytes subsequently produced are of two sorts : the one possesses 54 chromosomes and the other 55 (Figs. 35-36). In the secondary spermatocyte, it is difficult to distinguish the sex-chromosomes due to the absence of any character distinguishable from those of the autosomes. Further, there are no precocious chromosome in the second division.

In the following, the behaviour of the sex-trivalent will be traced in detail during the meiotic prophase stages. In the leptotene nucleus of the meiotic prophase the sex-chromosomes made their appearance distinguishable from the autosomes. In this stage, two compact chromatic masses which are positively heteropycnotic could be observed among lightly stained spireme threads in the nucleus. One of them is composed of two parts of nearly equal size: they connect with each other transversely. The other one is about 1/2 the size of the above double mass having no connection therewith (Figs. 29–30). In view of the heteropycnotic character in the leptotene stage and considered from their shape and size observed at metaphase, it seems most probable that the double mass consists of the X_1 and X_2 in association, whilst the smaller one is the Y. Since there is no nucleolus in any stage of the meiotic prophase, the association of the sex-chromosomes with the nucleolus was not observable. With the advance in the stage of the meiotic prophase, there appear many chromatic bodies in the nucleus indistinguishable in size from the X_1, X_2 and Y; this makes impossible the distinction of the sex-chromosomes from the autosomal chromatic bodies. Though there is no evidence, the establishment of the distance pairing of the sex-elements might have taken place in late diakinetic stage, as has been reported to occur in *Plethosmylus decoratus* (Hirai '56).

In conclusion the diploid number was found in the present species to be 109 forming a complex of 18V'+91R'. In the primary spermatocyte, 53 bivalents and the X_1X_2Y-trivalent were observed. There are two kinds of secondary spermatocytes having 54 and 55 chromosomes, respectively. During the meiotic prophase two X's and Y make their appearance as distinct chromatic bodies in the auxocyte. The X_1X_2Y-trivalent displays a distance pairing in the spindle of the primary spermatocyte metaphase. The sex-mechanism of X_1X_2Y-type as found in the present species is quite unique in the Crustacea, so far as the chromosome survey has gone.

8) *Eupagurus ochotensis* BRANDT

This species is a common hermit-crab showing a wide distribution in the shallow sea all around Japan. It belongs to the Family Paguridae, Paguridea, Anomura of the Order Decapoda. The materials of the present study were obtained in shallow waters adjoining the Akkeshi Marine Biological Station, Hokkaido by dredging in June, 1934.

Many dividing figures of spermatogonia were observed in the testes. The early spermatogonia were used for observation to avoid mistake in counting the number of chromosomes, since they are so large in number. Fig. 37 is an example of metaphase equatorial plates of early spermatogonia. The chromosomes constitute a large round equatorial plate distributing themselves well apart from one another with sharply defined contour. Overlapping of chromosomes occurs to a slight degree. In spite of their large number, therefore, counting of chromosomes is not very difficult. After careful counting, it was decided that the diploid complex consists of 254 chromosomes. The spermatogonial garniture is polymorphic in nature: of the 254 chromosomes, 18 are metacentric V-shaped ones while the remaining ones are all acrocentric rod- and short dot-shaped. Those 18

metacentric V-shaped chromosomes are sorted into 9 pairs by comparing their sizes and shapes. Each of them has a nearly submedian attachment of the spindle fibres. In the acrocentric rod-shaped chromosomes, it was impossible to sort the homologous pairs, except for several larger ones. As a whole, the larger chromosomes occupy a peripheral position in the equatorial plate surrounding the smaller one in the central portion.

Division of the spermatocytes takes place approximately simultaneously within a cyst. Many dividing figures, therefore, could be observed in the testis. The chromosomes of a primary spermatocyte constitute a round equatorial plate; they scatter well apart from each other (Figs. 38–39). Careful observations of many equatorial plates at metaphase made it clear that every metaphase plate contains 127 bivalent chromosomes without exception. All the bivalents take a dumbbell shape excepting some larger ones which appear as criss-cross tetrads. They may be the descendants of 9 pairs of metacentric V-shaped chromosomes in the spermatogonia. At anaphase, each of the bivalents divides synchronously into two equal halves. Dividing figures of the secondary spermatocytes are fewer than those of primary spermatocytes. Every secondary spermatocyte shows at metaphase 127 chromosomes of dyad nature constituting a circular equatorial plate (Fig. 40). There is only one kind of secondary spermatocytes. Several larger ones occur as the descendants of V-shaped chromosomes in the spermatogonia. In division, all the elements divide synchronously without showing any element of unusual behaviour.

In conclusion, the present species is characterized by the diploid number of 254 constituting a formula of 18V'+236R' and showing the haploid number of 127. This chromosome number is the greatest so far reported among animals.

9) *Coenobita rugosa H. MILNE-EDWARDS*

The present species is a terrestrial hermit-crab, living in southern Islands of the Japan. It belongs to the Family Coenobitidae, Paguridea, Anomura of the Order Decapoda. Specimens captured on Chichijima of the Bonin Islands in July, 1937, provided the material for study.

Though testes of several individuals were sectioned and above 200 preparations are investigated, there could be found no dividing figure except a solitary excellent metaphase plate of the early spermatogonia in the testes as given in Fig. 41. The equatorial plate is very large in dimensions; the chromosomes distribute evenly well apart from each other. This makes easy the counting of the chromosomes. After careful counting the number of chromosomes is decided as 230. They are all of acrocentric rod-shape showing gradual diminution of size from rod- to minute dot-shape. The present species has an isomorphic complex of chromosomes. It is rather difficult to sort the homologous pairs in the diploid complex. The present observations could not include any of haploid chromosomes, since there were no dividing figures in the testes. The chromosome number of the present species, 230, ranks second in the animal chromosomes so far as the scope of the present chromosome survey is concerned.

10) *Paralithodes camtschatica TILESIUS*

The present species is a well-known edible crab widely distributed along the coasts of the north Pacific Ocean. It belongs to the Family Lithodidae, Paguridea, Anomura of the Order Decapoda. The material consists of testes from several specimens captured by nets from the waters adjoining the Akkeshi Marine Biological Station, Hokkaido, in the middle part of May, 1934. They were operated and fixed immediately after capture on board the fishing boats.

The spermatogonia are found constituting the inner walls of the seminal tubules, the central portions of the latter being occupied by cells of various kinds in maturating stage. The spermatogonia do not undergo simultaneous division in a cyst, but dividing figures are encountered independently among the resting cells. As seen in the polar view of the metaphase, the chromosomes arrange themselves well apart from one another, forming a farily round equatorial plate. It is not very difficult, therefore, to count the number of chromosomes, in spite of their considerably large number. By careful counting of many clear plates, the diploid number was determined to be 208 (Figs. 42–43). The chromosomes range in length from rod to dot, showing gradual diminution in size. The larger rod-shaped chromosomes are sometimes curved. These chromosomes seem to be metacentric V-shaped ones but by comparison of several metaphase complexes, it was decided that all the chromosomes are acrocentric ones.

In the metaphase polar view of primary spermatocytes, the bivalents appear with distinct clearness, scattered evenly on the equatorial plate, as drawn in Figs. 44–45. In the present stage, therefore, the number of chromosomes can be counted with much ease and without mistake. By counting many clear metaphase plate, it was ascertained that every primary spermatocyte contains 104 bivalents without exception. The bivalents vary in size, and when viewed from the pole, the majority of the larger ones exhibit a clear dumbbell shape, each one showing a conspicuous transverse suture in the middle region. Because of this suture each bivalent seems to be composed of two equal halves. In the smaller bivalents, however, one can hardly demonstrate the actual presence of that middle suture. In the ensuing division, the bivalents are all separated equally into two sister components. The separation of the sister chromosomes is quite synchronous in every bivalent.

The symmetrical distribution of chromosomes in the primary spermatocyte division gives rise to only one kind of secondary spermatocyte in respect to the chromosome garniture. The metaphase equatorial plate of the secondary spermatocyte much resembles that of the primary spermatocyte, but is reduced in dimensions as compared with the latter. The individual chromosomes exhibit a distinct dual nature, they are scattered all over the equatorial plate without any overlapping with one another. In the equatorial plates examined, it is proved that the number of dyads is 104 without any exception (Figs. 46–47). Nothing particular is found in the mode of the secondry spermatocyte division; every dyad divides into two monads.

The chromosome number of the present species is 208 in diploid and 104 in haploid,

probably the same as in *Cambarus immunis* (?) in which Fasten ('14) reported 104 chromosomes as the haploid number. So far as the present observations show, in *Paralithodes camtschatica*, there are to be found no kinds of chromosomes, so particular in form and in behaviour as to be considered as sex-chromosomes.

***11) Paralithodes platypus* BRANDT**

This species is an edible crab, having a close relationship to the previous species, *Paralithodes camtschatica*. It is distributed along the coasts of the north Pacific Ocean together with the previous species. It belongs also to the Family Lithodidae, Paguridea, Anomura of the Order Decapoda. The crabs on which the present work was based were obtained by nets in the Okhotsk Sea adjacent to the Kurile Islands. The preservation of the testes was made through the courtesy of Mr. Isamu Takeuchi of the Hokkaido Regional Fisheries Research Laboratory, to whom the author wishes to express his cordial thanks.

The dividing figures are encountered independently among the resting spermatogonial cells. At metaphase, the chromosomes constitute a large circular equatorial plate well apart from one another. It is not so difficult, therefore, to count the number of chromosome notwithstanding their large number. Based on the counting of several clear plates, the diploid number was determined to be 206 (Fig. 48). This number is two less than that of the previous species, *Paralithodes camtschatica*. Remarkable difference between the chromosome garnitures of these two related species, is the existence of metacentric V-shaped chromosomes in the former species. In detail, the chromosome complex of *P. camtschatica* are isomorphic in nature, while *P. platypus* has a polymorphic complex : of 206 chromosomes, 18 are metacentric V-shaped, while the remaining 188 are acrocentric rod-shaped showing gradual diminution of size from long rods to minute dot. The 18 metacentric chromosomes are sorted into 9 homologous pairs according to their sizes and forms, though it is rather difficult to identify the homologous pairs in the 188 acrocentric chromosomes.

The primary spermatocytes divide synchronously in each cyst. In the metaphase polar view of this stage, the bivalents take their appearance with excellent clearness distributing themselves evenly on the equatorial plate (Figs. 49–50). There are 103 bivalents in every equatorial plate without exception. Of these 103 bivalents, several comparatively larger ones take dumbbell form whilst the remaining ones appear as a rectangular in form. The transverse suture across the central portion of each bivalent could not be ascertained in the present species though it was detected rather distinctly in the chromosomes of *Cambaroides japonicus* and *Paralithodes camtschatica*. It may depend on strong condensation of chromonema at this stage. At anaphase, all the bivalents separate equally into two daughter havles and go synchronously to each pole.

Careful counting of many equatorial plates of secondary spermatocytes shows without any exception 103 chromosomes of dyad nature (Fig. 51). Several of them are comparatively larger in size. They may be the descendants of 9 paris of V-shaped chromosomes as found in the spermatogonia. At anaphase, all the dyads separate equally into two equal halves

simultaneously. No chromosome with any particular characters could be detected throughout either of the reduction divisions.

It is evident from the above observations that the present species possesses 206 diploid chromosomes consisting of 18V'+188R' and 103 bivalents in haploid. The number, 206, is two less than those of the closely related species, *Paralithodes camtschatica*. The present species is characterized by a polymorphic chromosome garniture, but the latter by an isomorphic complex.

12) Matuta lunaris FORSKÅL

The present specizes is a common sea-shore crab distributed widely along the southern coasts of Japan from Sagami Bay to Formosa. It belongs to the Family Carapidae, Oxystomata, Brachyura, Reptantia of the Order Decapoda. Specimens capture on the seashore near the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in May, 1935 provided the material for the present study.

Dividing figures of spermatogonia are observed here and there among the resting spermatogonial cells, which constitute the inner walls of seminal tubules. The spermatogonial cells are very small when compared it with those of above described species belonging to the other groups of Decapoda : Palinura, Astacura and Anomura. The cells of the present species are 1/2 the diameter of those of *Paralithodes*. Accordingly the number of chromosomes contained in the cells of the present species is very small. After careful observations of several excellent metaphase plates, it was decided that the diploid complex consists of 94 chromosomes. When observed from the pole at metaphase, the chromosomes assume a circular rosette form in arrangement, as the larger chromosomes lie at the periphery with the smaller ones at the central portion of the equatorial plate. Examples are given in Figs. 52 and 53. As is recognizable in the figures, the spermatogonial garniture is of polymorphic nature. Four chromosomes of a metacentric V-shaped and 90 ones having an acrocentric nature, diminishing gradually in size from long rod to minute dot, constitute every spermatogonial garniture. Though the four metacentric chromosomes could easily be paired by their sizes and forms, it is difficult in the remaining 90 acrocentric ones to find the actual partners for pairing, since their size-difference is gradual.

The chromosomes of the primary spermatocytes are observed with absolute clearness in the polar view of the metaphase plate in spite of their small size, as they scatter evenly and are distinctly separated from each other. The number of bivalents contained in every equatorial plate is 47 invariably (Figs. 54–55). The larger ones are dumbbell in shape as commonly occurs in crustaceans. The smaller ones exhibit rectangular form. The transverse suture across the central portion of each bivalent could not be observed in the present species. At anaphase, all the bivalents separate into equal halves simultaneously.

Dividing figures of secondary spermatocytes were observable abundantly. As expected from the mode of separation of bivalents in the previous division, only one kind of secondary spermatocyte is produced in respect to the chromosome complex. Fig. 56 is an example of the metaphase polar view of the secondary spermatocyte which shows 47 chromosomes in

dyad nature.

Based on the above results, it is concluded that the chromosome number of the present species is 94 consisting of 4V'+90R' in diploid and 47 in haploid. No chromosome, peculiar in shape or in behaviour, was found in either meiotic division.

***13) Philyra pisum* DE HAAN**

The present species is a small crab, with a carapace about 20 m.m. in breadth. It is common in muddy shallow seas throughout Japan from Iwate Prefecture to Kyûshû. The present study was based on the testicular material derived from several specimens captured in Kisarazu, Chiba Prefecture in May, 1943. It belongs to the Family Leucosiidae, Oxystomata, Brachyura, Reptantia of the Order Decapoda.

Probably due to the unfavorable season the spermatogonial cells are very scarce in every testis. They were found in thin inner walls of the cyst, surrounding the cells in various stages of meiotic divisions and mature spermatozoa at the central portion of the cyst. Accordingly, the dividing figures of spermatogonia are scarce. Fig. 57 is an excellent example of the metaphase equatorial plates of spermatogonia. The dimensions of cells are also as small as those of the preceding species, *Matuta lunaris*. The chromosomes forming every equatorial plate are 114 in number. Four large metacentric chromosomes of V-shape appear in the periphery of the equatorial plate. They can easily be classified into two homologous pairs, but the remaining 110 chromosomes are all acrocentric chromosomes assuming a rod-shape, diminishing gradually in size from rod to dot shape.

The dividing figures of spermatocytes were abundant in the testes. Figs. 58 and 59 are the metaphase polar views of the primary spermatocytes. The bivalents constitute a circular equatorial plate, distributing themselves equally well apart from each other. In every equatoiral plate, 57 bivalents are counted without exception. All the bivalent take the form of a rectangle. Neither the transverse suture across the central portion of each bivalent nor the dumbbell shape, common for decapod bivalents, could be observed. In the ensuing division, all the bivalents separate equally into two equal halves.

There is only one kind of secondary spermatocytes as expected from the mode of the first division. In the equatorial plate of the secondary spermatocytes, 57 chromosomes of dayd character distribute evenly arranging themselves in the equatorial plate well apart from each other (Fig. 60). Among them, two chromosomes appear as a double V. They seem to correspond to two pairs of metacentric V-shaped chromosomes in the spermatogonia. The dyads separate equally into two monads at anaphase of the second division.

So far as the present observations show, in *Philyra pisum* there are discovered no special chromosome, so particular in form and in behaviour as to be considered the sex-chromosome. The present species is characterized by the diploid number of 114 having a complex of 4V'+110R' and the haploid number of 57.

***14) Ranina ranina* (LINNÉ)**

This is a species of large edible crabs with a carapace about 100 m.m. in breadth, being very famous for its characteristic form and beautiful colouration on the carapace. It

distributes widely from Sagami Bay to the coast of the south Pacific Ocean. The specimens which the present material was based were captured by traps in the waters adjoining the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in September, 1936. It belongs to the Family Raninidae, Oxystomata, Brachyura, Reptantia of the Order Decapoda.

The spermatogonia do not undergo a simultaneous division in a cyst: the dividing figures are found independently together with the resting cells. Due to favourable season of collection many dividing figures could be observed in the material. The cell diameter of the present species is comparatively larger than that of the above two species of Brachyura, *Matuta lunaris* and *Philyra pisum*.

The spermatogonial chromosomes assume a rosette form in their metaphase arrangement, as they lie radially against the centre of the equatorial plate, being well-apart from one another with sharply defined contour. Example are given in Fig. 61. Spermatogonial complex is polymorphic in nature containing 106 chromosomes in each. Of them, 24 are metacentric elements of V-shape and the remaining 82 are acrocentric rod-shaped ones. The latter chromosomes vary in shape from long rod to minute dot. According to the sizes and shapes, all the chromosomes are cleary sorted into 53 homologous pairs.

The metaphase equatorial plate of the primary spermatocytes is very large having nearly the same diameter as that of the spermatogonium. The bivalent chromosomes, 53 in number, are observed with extreme clearness in the equatorial plate (Fig. 62). They are arranged in a radial manner distributing well apart at nearly equal distance from one another. As usual the larger bivalents take a peripheral position with those of smaller size in the central region of the spindle. Some of the larger bivalents take a strange form in their morphology. Under close observation it becomes evident that they are a modification in shape of the simple criss-cross tetrad. The other bivalents excepting the above larger ones, have the shape of a dumbbell or rectangle. The bivalents of the former shape show no remarkable suture in their middle region. At anaphase, all the bivalents separate into two equal halves simultaneously.

Actually there is only one kind of secondary spermatocytes in respect to the chromosome complex. Fig. 63 is an example of the polar view of the secondary spermatocyte metaphase. There are 53 dyads without exception in every plate studied. The chromosomes are quite similar in arrangement to those of the spermatogonium and primary spermatocyte, as the larger dyads are arranged in periphery of the spindle while the dot-like ones are in the central region. Further, all chromosomes corresponding to those of the spermatogonium are detected again in the garniture of the secondary spermatocyte: there are 12 metacentric V-shaped chromosomes and the remaining acrocentric ones varying in shape from rod to dot. At anaphase of the second division these dyads divide synchronously into two equal halves.

No chromosomes peculiar in form and shape could be detected throughout the two reduction divisions. The chromosome number of the present species is 106 in diploid and

53 in haploid in the male. The formula is represented by 24V'+82R'.

15) *Macrocheira kaempferi* DE HAAN

The present species is well-known for being very large in size among crabs. They are deep-sea dwellers in Japan. The species belongs to the Family Majidae, Oxyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The crabs, from which the material for the present work was obtained, were collected in April, 1937 from Suruga Bay, to the west of Izu Peninsula, at depths of 200 to 400 meters.

Dividing figures of spermatogonia were abundant in the testes among the resting spermatogonia. Two representations of the metaphase plates are shown in Figs. 64 and 65. Every metaphase equatorial plate shows 106 chromosomes which arrange themselves well apart from one another. Of them, 16 are metacentric V-shaped elements and the remaining ones are of acrocentric type diminishing gradually in size and shape from rod to dot. Those 16 metacentric chromosomes and several of the larger acrocentric ones can be arranged in homologous pairs by their shapes and sizes. As to the shorter rod- and dot-shaped ones, however, it seems impossible to know the actual partners for homologous pairing.

The dimensions of the equatorial plate of primary spermatocytes are smaller than those of the previous species, *Ranina ranina*, but they are greater than those of *Matuta lunaris* and *Philyra pisum*. In diameter it measures about 1/2 that of the former species and 3/2 times that of the latter two species. The bivalents arrange themselve equidistantly on the equatorial plate, showing no overlapping of chromosomes (Fig. 66). In all plates examined, 53 bivalents are observed without excpetion. Several of the larger bivalenets assume the form of a criss-cross. They may be the descendants of metacentric V-shaped chromosomes in the spermatogonia. The other smaller bivalents are dumbbell or rectangular in shape. The transverse suture across the middle region of each chromosome is obscure in the bivalents of the present species. In the ensuing division, all the bivalents separate into equal halves synchronously. At the circumference of the equatorial plate, there are always about ten or more black bodies nearly similar in size. They were found surrounding the equatorial plate having no relation to the spindle fibre (Fig. 67). These bodies were also found outside the meiotic prophasic nuclei lying close to the nuclear membrane. It seems most probably from the above facts that these bodies are not chromosomes but may be chromatoid bodies. As seen in Fig. 67, all the bivalents separate into two equal halves synchronously at anaphase.

At the second metaphase the chromosomes form a circular equatorial plate distributing themsevles evenly in it. They are 53 in number and dyad in nature (Fig. 68). There are 8 x-shaped dyads. They may be the descendants of the 8 pairs of V-shaped chromosomes of the spermatogonia. The remaining 45 elements are all acrocentric rod-shaped. The complex of the secondary spermatocyte is just half the spermatogonial complex. At anaphase, the 53 dyads each separate into two equal halves.

During both meiotic divisions, there are to be seen no chromosomes having any peculiar characters in form and in shape. In conclusion the present species is characterized by the chromosome number of 106 in diploid and 53 in haploid in the male. The present species is the same in chromosome number as *Ranina ranina*, but it shows a different complex. The complex consists of 16V'+90R'

***16) Ovalipes punctatus* (DE HAAN)**

The present crab is yellow in colouration with dark spots scattered all over the carapace which is about 56 m.m. in breadth. It is distributed widely in Japan from Hokkaido to Ryûkyû, belonging to the Family Portunidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The specimens which furnished material for study were obtained by means of dradging at Kakisaki, Shimoda Bay, Izu Peninsula, in May, 1936.

Dividing figures of the spermatogonia are rather few in number. Figs. 69 and 70 show the metaphase equatorial plates of the spermatogonial divisions. Counting of the chromosome number is rather easy. It showed that the diploid complement consists of 103 chromosomes, of which 4, or 2 pairs, are metacentric V-shaped while the remaining ones are all acrocentric of short rod-shape. The occurrence of the odd number of spermatogonial chromosomes suggests the presence of an unpaired element in this species. But it was quite impossible to detect such an unpaired element in the diploid complex, because of the fact that the 99 acrocentric rod-shaped chromosomes gradually diminish in size from short-rod to minute dot, there being no remarkable characteristics in any chromosomes beyond the length.

The meiotic divisions are found occurring simultaneously in several cysts in the testes. Over one hundred metaphase figures of the primary spermatocyte were observable in a single section. The dimensions of the equatorial plate of primary spermatocyte at metaphase are slightly smaller than those of the preceding species, *Macrocheira kaempferi*. A metaphase polar view shows 51 distinct bivalents which constitute a beautiful circular equatorial plate. The majority of them assume a rectangular or dumbbell shape which is common in the chromosomes of many crustancean species. By changing the focus of the microscope from equatorial plate a little upward or downward, the existence is disclosed of a chromosome which lies in a nearly central portion of the equatorial plate situated always in one side of the latter (Figs. 71–72). Viewed from the side of the equatorial plate, the presence of this peculiar element becomes very evident, being slightly apart from the equatorial plate in which other elements lie, as clearly shown in Figs. 73 and 74. After close examinations, it becomes certain that this particular chromosome is completely present within the spindle of the metaphase. In shape this chromosome is apparently more slender than the ordinary bivalents; it tapers at one end directed toward the equatoiral plate. Considered from its structure, this chromosome is certainly of a dyad nature and not a bivalent. At anaphase, this chromosome does not separate at all and migrates towards one of the poles of the spindle. As a result of this division, one of the daughter complexes

produced contains this element in its complement. From the fact shown in the morphology and behaviour as above observed, it is quite natural to consider that this chromosome having a dyad nature is the X-chromosome, just comparable to that one generally found in Orthopteran insects. Further, the occurrence of the odd number of spermatogonial chromosome confirms the presence of a single X-chromosome. It is remarkable that the X-chromosome always lies in a central position of the spindle. Thus the chromosome constitution of the primary spermatocyte of this species is shown by the formula; $n=51+X$.

The secondary spermatocyte metaphase shows chromosomes with dyad nature scattered evenly in a circular equatorial plate. Careful counting of the number indicates that there are two kinds of cells in the secondary spermatocyte with regard to the number of chromosomes. One of them shows 51 chromosomes (Figs. 75–76), while the other 52 (Figs. 77–78). Considered from the distribution of the X-chromosome as it occurred in the primary spermatocyte division, it is apparent that the cell having 52 elements involves in it an X-chromosome. The X element is entirely indistinguishable in the complex for the reason noted in the case of the spermatogonial cell. At anaphase, all the elements segregate synchronously, and as the result there are produced two kinds of spermatids, one with the X and the other without it. Hence the chromosome complexes of the secondary spermatocyte are as follows; (a) no-X-class having 51 chromosomes, and (b) X-class having 52 chromosomes.

In summary, the chromosome constitution of the present species is shown by the formula; $2n$, $103=4V'+98R'+X$. and $n=51+X$. The X–O type of sex-mechanism of the present species is the first to be found in the decapod Crustacea, although the X-Y and the X_1X_2-Y sex-mechanisms were found to occur in several other decapods.

17) *Scylla serrata* (FORSKÅL)

This species is a large edible crab, 197 m.m. in breadth of carapace and a member of the Subfamily Lupinae, Portunidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. It is distributed widely from Sagami Bay to the coasts of the Indo-Pacific region. The material upon which the present study was based was obtained from shallow waters near Nabeta, Shimoda Bay, Izu Peninsula, in September, 1936.

The present species, together with the preceding *Ovalipes punctatus*, are included taxonomically in the same Family Portunidae, though they belong to different subfamilies: the present species belongs to Lupinae, while the latter to Portuninae. The cells of the present species are smaller in size than those of the latter species. They are nearly the same size as those of *Matuta lunaris* of the Family Carapidae, Oxystomata.

The spermatogonia do not undergo simultaneous division in a cyst, but dividing figures are encountered independently among the resting spermatogonial cells. Two kinds of spermatogonia, the early and late may be classified by their size; the former has twice the size of the latter. Fig. 79 is an example of early spermatogonia and Fig. 80 is that of the late ones. The size-relation of the chromosomes is parallel to the cell-size. The spermatogonial chromosomes are of isomorphic nature, since all the chromosomes are acrocentric rod-

shaped, diminishing gradually in size from long rod to minute dot. The counting of the chromosome number shows that the diploid complement consists of 106 chromosomes. The chromosome number of the present species is just identical with that of the next preceding two species, *Ranina ranina* and *Macrocheira kaempferi*, though they differ constitution from each other in their chromosome complexes.

Division of the primary spermatocyte occurs symultaneously within a cyst. Many dividing figures, therefore, could be observed in the same sections. Bivalents constitute a beautiful circular equatorial plate at metaphase (Figs. 81–82). In a complex, 53 bivalents, all having rectangular shape, arranged themsevles evenly on the equatorial plate. They each separate synchronously into two equal halves at anaphase. Since the present species belongs to the same Family Portunidae with the previous species, *Ovalipes punctatus* having the X-O sex-mechanisms, the primary spermatocytes of the present species were fully investigated. No particular chromosome to be considered as the sex-chromosome, however, was found in respect to behaviour or in morphology.

The dividing figures of the secondary spermatocyte were abundant among the primary spermatocytes. In dimensions the equatorial plate of the secondary spermatocyte is very much smaller than the primary spermatocytes. As expected from the mode of the first division, there is only one kind of secondary spermatocytes. As seen in Fig. 83, 53 chromosomes of dyad nature could easily be counted in every equatorial plate. They are all of rod- or dot-shape. At anapahse, they separate into two equal halves. Also no chromosome peculiar in form or behaviour could be observed in the second division.

In fine, it is concluded that the chromosome number of the present species is 106 in diploid and 53 in haploid.

18) Telmessus cheiragonus (TILESIUS)

This is a brown crab having short bristles all over the carapace which is about 80 m.m. in breadth. It is distributed widely from Hokkaido to California along the coasts of the North Pacific. It belongs to the Family Atelecyclidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The specimens which provided the material for the present study were captured in shallow waters at Muroran, Hokkaido in April, 1940.

Dividing figures of spermatogonia were encountered abundantly in a testis scattering here and there among the cells of resting spermatogonia. Figs. 84 and 85 show examples of the metaphase polar views. The chromosomes constitute a circular equatorial plate arranging themselves well apart from one another. By careful counting of many clear plates, the diploid number was determined to be 124. In the peripheral portion of the equatorial plate, 8 metacentric V-shaped ones are observable, though occasionally some of the smaller V-shaped ones are found lying at the central region. According to their sizes and shapes these 8 metacentric chromosomes are sorted into 4 homologous pairs. The remaining 116 chromosomes are all acrocentric in nature, diminishing in size from rod to dots. It seems impossible to arrange these acrocentric chromosomes into homologous pairs, since

there is no distinguishing character beyond length.

The first division occurs synchronously in a cyst. Many dividing figures could be observed in the same cyst. The metaphase equatorial plate is about 3/2 times larger than those of the preceding species, *Scylla serrata* in diameter. Every equatorial plate contains 62 bivalents which are well-apart from one another. Larger bivalents take the form of a dumbbell and smaller ones appear rectangular in form. Among these dumbbell shaped bivalents there are a few larger ones which are considered to be the descendants of metacentric V-shaped chromosomes of spermatogonia. They are not very remarkable in appearance, since the size-difference between the smaller V-shaped chromosomes and longest acrocentric rod-shaped ones is not very evident (Figs. 86–87). At anaphase, all the bivalents separate simultaneously into two equal daughter halves.

In cysts next to the primary spermatocyte at division, the second division took place abundantly. In spite of their small size, 62 chromosomes of dyad nature could be easily observed without any doubt at metaphase (Fig. 88). Four dyads are observed having metacentric structure, while remaining 58 are acrocentric rod-shaped chromosomes. At anaphase, separation of all dyads occurs synchronously. Throughout the two meiotic divisions, no chromosome with any peculiar behaviour could be detected.

Based on the above results, it is evident that the chromosome number of the present species is 124 in diploid and 62 in haploid. The diploid complex is formulated as 8V'+116R'.

19) *Atergatis floridus* LINNÉ

This is a common crab, about 70 m.m. in carapace breadth, inhabiting the rocky sea-shores. It is of the Family Xanthidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. It shows a wide distribution form the Bôsô Peninsula, Japan, to the coast of the Indo-Pacific region. The crabs, from which the material of the present study was obtained, were collected from shores near the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in November, 1936.

Due probably to the period of collection of the material, almost all the cysts of testes are filled with mature spermatozoa, while spermatocytes in process of reduction division are observed in the small portion of a testis. The dividing figures of spermatogonia are found very rarely. Fig. 89 is the most ideal metaphase equatorial plate so far observed. The chromosomes distribute themselves well apart from one another, overlapping of chromosomes occurs to a slight degree, though they do not show circular metaphase arrangement. There are 104 easily countable chromosomes in this plate. The spermatogonial complex is of polymorphic nature; of the 104 chromosomes, 4 are metacentric V-shaped elements, while the remaining ones are all acrocentric rods showing gradual diminution in size. Those 4 metacentric chromosomes and several of the longer rod ones can be arranged in homologous pairs, by their shaps and sizes. However, it was impossible to find the actual partners among the remaining shorter chromosomes, for there are many

chromosomes having a similar length.

Since the division occurs synchronously in a cyst, primary spermatocytes at metaphase can be observed abundantly. Bivalents are observed with absolute clearness in polar view of every metaphase plate, as they scatter evenly. The dimensions of the metaphase plate are nearly identical with those of *Telmessus cheiragonus*. Examples are given in Figs. 90 and 91, in each of which there are contained 52 bivalents. Two of them are larger than the other ones in size, taking the form of a criss-cross or modification of it. They may correspond with the metacentric V-shaped chromosomes of the spermatogonial cell. The other 50 bivalents take the shape common for those of Decapoda : dumbbell and rectangle. All the bivalents divide synchronously into two equal daughter halves at anaphase.

The symmetrical distribution of the bivalents in the first division gives rise to only one kind of secondary spermatocytes in respect to the chromosome complex. The individual chromosomes of the second metaphase exhibit a distinct dual nature and are scattered all over the plate without any overlapping of chromosomes. Fig. 92 is an example of metaphase equatorial plates. Counting shows that the number of dyads is 52. Nothing particular is found in the mode of the present division ; every dyad divides into two equal daughter monads.

It is concluded that the chromosome number of the present species is 104 in diploid and 52 in haploid. The complex is shown by 4V'+100R'. There are no chromosomes particular in form and behaviour during meiotic divisions.

***20) Pachygrapsus crassipes* RANDALL**

This is also a crab commonly found on rocky sea-shores along the coasts of Japan from Hakodate, Hokkaido to Kyûsyû, having carapace 33.5 m.m. in breadth. It is a member of the Subfamily Grapsinae, Grapsidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. The crabs with which the present study was carried out were captured near the Mitsui Institute of Marine Biology at Suzaki near Shimoda, Izu Peninsula in July, 1936.

The spermatogonia do not undergo simultaneous division, being found intermingled with resting cells. At metaphase the chromosomes constitute a circular equatorial plate showing no overlapping of chromosomes. An example is shown in Fig. 93. After careful counting of many equatorial plates of spermatogonia, it was concluded that the diploid number of chromosomes is 118. The diploid chromosomes complex is of isomorphic nature : all the chromosomes are acrocentric in attachment and vary in length from short rod to minute dot. It seems impossible to identify the homologous pairs, because they diminish gradually in size with slight differences.

Since the division of primary spermatocytes takes place synchronously within one cyst, many dividing figures were found in favorable condition for study of the chromosomes. Figs. 94–96 are examples of metaphase polar views of the primary spermatocytes. The dimensions of the equatorial plate are nearly similar to those of *Atergatis floridus*.

There are 58 bivalent distributed evenly in all parts of the plate. Size differences are not very remarkable among them. Every bivalent exhibits a median transverse suture in accordance with the cases previously observed in different kinds of Decapoda : e.g., *Cambaroides japonicus*, *Paralithodes camtschatica*, *Panulirus japonicus*, etc. Closer examination made it clear that every individual complex of the chromosomes involves two more elements in addition to the 58 bivalents mentioned above. The additional two elements do not lie at the same level with the 58 bivalents, therefore being entirely invisible so long as attention is directed to that level. They are first evident only when the focus is changed gradually upward or downward from where the bivalents lie, since they take their position separately on the two sides of the equatorial plate. When one of them is visible under a certain focus the other one is completely hidden from vision, and *vice versa*. It is of particular importance that two such chromosomes are found constantly in every cell of the same dividing phase. To examine their morphology in detail, attention is particularly called to the side view of the spindle, of which three examples are reproduced (Figs. 97–99). As is readily recognizable from these figures, the two chromosomes in question are not bivalents. They never appear as dumbbell or rectangular in form, but are evidently elements of rod-type, univalent in nature. They differ from each other in their relative magnitude, one being approximately 2/3 as long as the other one ; this ratio seems to be invariable. They lie in the central position of the plate, lying probably on a line connecting the two poles of the spindle. At the confronting end they become gradually tapered and more or less pointed at tips, while the opposite ends are rounded. In all the metaphase plates observed, these two distinct chromosomes are situated always considerably off the equatorial plate without exception. Such a structure and situation strongly support the view that these two distinct chromosomes might have been connected distantly together at the pointed ends to establish a distance pairing, as occurred in the sex-trivalent found in *Cervimunida princeps*. At anaphase, they separate from each other and migrate to each pole of the spindle with the daughter halves of ordinary bivalents.

From the facts stated above, these two particular chromosomes are nethier, bivalents displaced by some mechanical causes, nor chromatoid bodies, but the sex-chromosomes consisting of X and Y, in distance pairing, the X being represented by the larger one while the Y by the shorter one, in likeness to those commonly found in other kinds of animals. For example, the X and Y chromosomes in distance pairing were reported in a male Neuropteran, *Plethosmylus decoratus* by Hirai ('56). Oguma and Asana ('32), Kichijo ('34) and Naville et de Beaumont ('33) also found in some Neuropteran insects the X-Y chromosomes of a similar nature, though they indicated that the situation is the result of precession of the two elements. Based on the above results it is evident that the present species possesses an X-Y sex-determining mechanism in the male. Thus the chromosome constitution of the primary spermatocyte is represented by $58+XY=59$; this is the actual haploid number occurring in the present species.

In the metaphase polar view of the secondary spermatocyte, chromosomes of dual

nature constitute a fairly circular equatorial plate distributing themselves well apart from one another. An example is shown in Fig. 100. There are 59 chromosomes in every equatorial plate under study. There should be present two kinds of secondary spermatocytes, of which one contains the X chromosome and the other involves the Y. But, the distinction of the two particular elements is practically impossible, since the sex-chromosomes have no characteristic shape nor dimension for distinguishment from the autosomes. Further, at anaphase, all dyads including X and Y chromosomes separate sychronously into two equal monads.

Based on the results of the above study, it is evident that the diploid number is 118 in the male of the present species, and that the primary spermatocyte shows 58 bivalents and an X-Y bivalent. Every secondary spermatocyte possesses 59 dyads. The XY sex-mechanism as found in the present species is a new type of sex-determination in the Crustacea, in contrast to the previously described two types: X_1X_2-Y type in *Cervimunida princeps* and X-O type in *Ovalipes punctatus*.

21) Hemigrapsus sanguineus (DE HAAN)

The present species is a common sea-shore crab having a brown carapase about 28m.m. in breadth. It is distinguished from the following species, *Hemigrapsus penicillatus*, since it has yellow strips on the legs and reddish spots on the chelae. It is distributed widely along the coasts of all Japan. It belongs to the Subfamily Varuninae, Grapsidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. Together with *Pachygrapsus crassipes* the present species belongs to the same Family Grapsidae, but to different subfamilies; the present species is a member of the Varuninae while *Pachygrapsus* belongs to the Grapsinae. The materials for the present study were obtained from some specimens captured along the sea-shore in the vicinity of Oshoro near Otaru, in September, 1937.

Many dividing figures of spermatogonia could be observed scattered amongst cells in the resting stage. The chromosomes constitute a circular metaphase plate arranging themselves evenly apart from each other (Figs. 101–102). By careful counting of many excellent metaphase plates, it was decided that the diploid number of chromosomes is 128, all having acrocentric fibre attachment. Since they gradually diminish in size from short rod to dot shape, it was rather impossible to identify the homologous pairs among them.

All the primary spermatocytes within a cyst divide rather synchronously. The dimensions of the metaphase plate are nearly similar in size with those of *Pachygrapsus crassipes*. As seen in Figs. 103–104, there are in the metaphase polar view 63 bivalent chromosomes which were distributed evenly throughout the whole area of the plate. Size-difference exists in a slight degree among them. They all have a dumbbell shape, but the median transverse sutures are observable only in those of the larger size. As was found in the previous species, *Pachygrapsus crassipes*, the present species is also characterized by the presence of two particular chromosomes. Each one of them lies on two sides of the

metaphase plate maintaning a definite distance from the latter. It is possible to catch their images only when the foci are changed from the equatorial plate. They take the central position in the metaphase plate and show a marked difference in their relative sizes. The smaller element is about one-third the larger one in length. The magnitude of the Y chromosome seems to be comparable to that of the smallest chromosome in the spermatogonial garniture (Figs. 103–104). The X is a slender rod-shaped chromosome, tapering at one end toward the Y. The distance between these two chromosomes is remarkably larger than in the preceding species, as seen in the metaphase side view (Figs. 105–106). These particular chromosomes are adequately explicable only by considering them as an X-Y complex in a distance pairing. At anaphase, they separate from each other and migrate to opposite poles together with daughter halves of ordinary bivalents. On this basis, the chromosome formula is formulated by $63+XY=64$, representing the haploid number of the present species.

Every equatorial plate of the secondary spermatocyte consists of 64 chromosomes of dyad nature, much reduced in size (Figs. 107–108). There are presumably present two kinds of chromosome complex with regard to the kind of sex-chromosome, irrespective of the same number of chromosomes. In other words, two kinds of secondary spermatocytes, X-class and Y-class, should be produced. But the distinction of these two kinds is impossible, so long as the X- and the Y-chromosomes are difficult of differentiation from others.

It is concluded from the above observation that the present species has 128 chromosomes of rod-type in diploid, and that the chromosome constitution of the primary spermatocyte is represented by $63+XY=64$ as the male haploid number. The present species is characterized by a distance pairing of the X-Y chromosomes in the male, as was found also in the previous species, *Pachygrapsus crassipes*.

22) *Hemigrapsus penicillatus* (DE HAAN)

The present species bears a close resemblance to the previous species, *Hemigrapsus sanguineus* in the general morphology, but is easily distinguishable from the latter by the following three points : 1) having tufts of long brown hairs on its chelae in the male, 2) no reddish spots on its chelae, and 3) no yellow strips on its legs. It belongs also to the Subfamily Varuninae of the Family Grapsidae, Brachyrhyncha, Brachygnata, Brachyura, Reptantia of the Order Decapoda. It is distributed along the coasts of all Japan. The specimens for this study were captured in shallow sea-water not far from the Mitsui Institute of Marine Biology at Suzaki, near Shimoda, Izu Peninsula, in March 1936.

Divisions of spermatogonia do not occur simultaneously within a cyst, dividing cells being found intermingled with the resting cells. Two kinds of spermatogonia, the early and late, may be classified only by their sizes. Fig. 109 is an example of an excellent spermatogonia in which chromosomes arrange themselves well apart from one another without overlapping. The counting of chromosomes, therefore, was very easy in spite of their large number ; it showed that the spermatogonial complex consists of 138 chromo-

somes. The diploid garniture is of polymorphic nature ; all the chromosomes are acrocentric rod-shaped, except only one pair, each of which is metacentric V-shaped. They show submedian attachment. There is difficulty in the identification of the homologous pairs in the acrocentric elemensts except for the longest ones which are remarkable by having slightly curved shape.

Dividing figures of primary spermatocytes could be observed abundantly within a cyst. Every metaphase equatorial plate contains 68 bivalents of dumbbell and rectangular shape (Figs. 110–111). In a few of the larger bivalents, median transverse sutures are distinct. Careful observation of many metaphase plates in both polar and side views made it clear that the present species is also characterized by an X and Y sex-mechanism as was found in the previous two species, *Pachygrapsus crassipes* and *Hemigrapsus sanguineus*. As seen in Figs. 112 to 114, the sex-chromosomes in distance pairing take their position at nearly the central position of the equatorial plate lying considerably off from the latter. The distance between the X and Y chromosomes is somewhat shorter than in *Hemigrapsus sanguineus*. The magnitude of the Y chromosome seems to be comparable to that of the third, or fourth smallest chromosome in the spermatogonial garniture. The Y chromosome is about one half the X chromosome in magnitude. The X chromosome is not slender but rather plump, tapering at one end toward the Y. At anaphase, the X element separates from the Y and they migrate to each pole with daughter halves of ordinary bivalents. The chromosome formula of the primary spermatocyte is therefore represented by 68+XY=69 that is the haploid number of the present species.

Dividing figures are rather scanty in the secondary spermatocyte. They are found near the cysts of primary spermatocytes in process of division. There are 59 chromosomes having distinct dyad structure in every metaphase plate (Fig. 115). It was actually impossible to distinguish two kinds of chromosome complex of secondary spermatocytes in respect to sex-chromosomes, because there are many chromosomes having nearly equal size which makes difficult the identification of the X and the Y chromosomes in the garniture.

Based on the above results the conclusion was possible that the present species is characterized by 138 chromosomes in the diploid complex which shows 2V'+136R', and that the primary spermatocyte complex is shown by 68+XY=69. The XY complex is remarkable by showing a distance pairing at the first metaphase, as occurred likewise in two species of the Family Grapsidae.

23) *Eriocheir japonicus* DE HAAN

The present species is a common crab distributed all over Japan as an inhabitant of fresh or brackish waters in rivers, streams or river mouths. It is characterized by carapace 62 m.m. in breadth and long brown tufts of hairs on both its chelae. It is also a member of the Subfamily Varuninae together with the preceding two species of *Hemigrapsus*. The crabs from which the material of the present work was secured, were

collected from shores near the Mitsui Institute of Marine Biology, Suzaki near Shimoda, Izu Peninsula in May, 1936.

Dividing figures of spermatogonia are found along the walls of cysts. In the metaphase polar view, the chromosomes appear very clearly as shown in Figs. 116 and 117. By their sizes, the early and late spermatogonia may be distinguishable (Figs. 116 early spermatogonium ; Fig. 117, late spermatogonium). In every equatorial plate examined, 148 chromosomes are invariably counted, showing the diploid number of the present species. The spermatogonial garniture is isomorphic in nature : all the chromosomes assume short rod-shape or spherular form having the acrocentric attachment of the spindle fibres. They vary in size slightly. Accordingly, it is vain to attempt to find the homologous pairs among the complex by means of comparison of their sizes.

The dividing figures of the primary spermatocyte could be observed abundantly in a certain cyst. The equatorial plate in diameter shows similar dimensions to the two species of *Hemigrapsus*. An equatorial plate is composed of 73 bivalents distributed evenly throughout the whole area of the plate, as shown in Figs. 118 to 119. The size difference is developed in small degree among these bivalents as in the case of two species of *Hemigrapsus* but the median transverse sutures are also observable in those of bivalents comparatively larger size. It is worthy of notice that in this species, the two peculiar chromosomes are to be discovered again in both sides of the metaphase plate maintaining definite distance from the latter. They take also the central position in the metaphase plate and show a marked difference in their relative size. The smaller one is about one-half the larger one in length. Sometimes, a constriction appears at the middle portion of the larger one of these chromosomes which tapering at one end toward the smaller one. The distance between the two particular chromosomes is less than in the former two species of *Hemigrapsus*, aud nearly identical with *Pachygrapsus crassipes*, a fact which the side view of metaphase plates obviously shows (Figs 120–121). These particular chromosomes in question should also be properly considered as the X and Y showing a distance pairing. The magnitude of the Y chromosome is comparable to those of the smallest or nearly the smallest chromosomes in the spermatogonial garniture. The X-element separates from the Y at anaphase and they migrate to each pole. It is evident from the above observations that the chromosome formula of the primary spermatocyte is represented by $73+XY=74$, which is the haploid number of the present species.

Every equatorial plate of the secondary spermatocytes shows 74 chromosomes of dyad nature, quite small in size (Fig. 122). There are presumably present two kinds of secondary spermatocytes with regard to in view of the kinds of the sex-chromosomes. But the distinction of two kinds is absolutely impossible, so long as the identification of the X and the Y chromosomes remains in obscurity.

The results of the present observations of this species are summarized thus : the number of chromosomes is 148 in diploid and 74 in haploid in the male, and the present

species is also characterized by the presence of the XY-mechanism. It is interesting to note that the XY-mechanism was found very clearly in several species of the Family Grapsidae.

24) *Gaetice depressus* (DE HAAN)

The present species is a common small sea-shore crab, common along the Pacific coast of Japan from Muroran, Hokkaido to Kyûsyû. It has a flat carapace of 25 m.m. breadth. It is also a member of the Subfamily Varuninae of the Family Grapsidae with the preceding three species. The material consists of the testes from four specimens collected from shallow sea-water near the Mitsui Institute of Marine Biology, at Suzaki near Shimoda, Izu Peninsula in April, 1937.

As the spermatogonial cells are very scanty in the testes, dividing figures also are very rare. A few polar views at metaphase could fortunately be obtained. Fig. 124 is an example of them. After careful counting of the plate, it was decided that the spermatogonial complex contains 152 chromosomes; 12 are metacentric V-shaped showing 6 homologous pairs, while the remaining 140 are all acrocentric rod-shaped ranging gradually in sizes from long rod to minute dot. The spermatogonial garniture is therefore polymorphic in nature.

In contrast to the spermatogonial cells, primary and secondary spermatocytes are found abundantly in the testes. Since the divisions took place synchronously in those contained within a cyst, many metaphase equatorial plates of primary and secondary spermatocytes could be observed. Figs. 125 and 126 are equatorial plates of primary spermatocytes at metaphase. The dimensions of the plate are nearly equal in diameter with those of the preceding species, *Eriocheir japonicus*. In the metaphase polar view 75 bivalents of dumbbell and rectangular shape distribute themselves equally well apart from one another to constitute a beautiful circular equatorial plate. In some of the larger bivalents appears a median transverse suture. By changing the focus of the microscope gently upward or downward from the equatorial plate where the bivalents lie, one may see two univalent chromosomes differing in sizes from one another. These two particular chromosomes may be considered as X and Y sex-chromosomes. It is of particular importance that the X and the Y are found constantly in the same condition at metaphase being considerably apart from the equatorial plate, lying nearly in the central portion of the equator (Figs. 127–128). The Y chromosome is about 1/2 the X in length. The magnitude of the Y chromosome seems to be comparable to that of the sixth or seventh grade larger than the very smallest size in the spermatogonial garniture. The X chromosome is rather plump in form, tapering at the one of the two ends toward the Y. The distance between the X and the Y is nearly identical to that in *Hemigrapsus sanguineus*. At anaphase, the two separate from each other and migrate to each pole of the spindle with daughter havles of ordinary bivalents. The chromosome formula of the primary spermatocyte is therefore represented by 75+XY=76, as the haploid complex of the present species.

As easily expected from the mode of division in the primary spermatocyte, there should be two kinds of secondary spermatocytes: one containing the X, aud the other the Y. As a matter of fact, however, the distinction of these two kinds was not successful because there are many chromosomes having nearly equal sizes to the X and the Y. In the metaphase polar view of secondary spermatocytes, the chromosomes having dyad nature constitute a circular equatorial plate distributing themselves evenly well apart from one another (Fig. 129). There are 76 dyads in every equatorial plate studied. Among them, several elements were observed as having x-shape. They are obviously the descendants of the V-shaped chromosomes in the spermatogonial garniture. At anaphase they separate into two equal monads simultaneously.

The conclusion was reached from the above investigation that the diploid complex of the present species contains 172 chromosomes showing 12V'+160R', and that the haploid number is 76 involving an XY sex-mechanism in the male. The fact is remarkably that the studied species of the Family Grapsidae possess the XY sex-mechanism without exception.

25) *Sesarma intermedia* (DE HAAN)

The present species is a half-terrestrial crab, dwelling in swamps and wet places near the sea coast. It measures 32 m.m. in carapace breadth and is distributed widely along the coasts of Asia from Tokyo Bay to the Indo-Pacific region. The crab is a member of the Family Grapsidae, belonging to the Subfamily Sesarminae. The crabs, from which the materials for the present work was obtained, were collected from the bank of a small stream running near the Mitsui Institute of Marine Biology, at Suzaki near Shimoda, Izu Peninsula at various times from March to July 1935. Though preparations were made in large number with these materials, there were found no dividing figures of spermatogenesis except only one which is shown in Fig. 123. In all testes observed, cysts are surrounded with thin layers of spermatogonia in resting stage and the central portions of cysts are almost empty or contain only a small number of mature spermatozoa.

The spermatogonial metaphase plate contains 102 chromosomes having acrocentric structure. Since these chromosomes diminish gradually in size from rod to minute dot, it seems impossible to identify the homologous pairs. Accordingly, it was difficult to deal with the X and Y chromosomes, although such elements were found to occur in five related species of the Family Grapsidae.

The present observations resulted in determining the chromosome number of the present species as 102 in diploid in the male. The existence of the sex-chromosomes remains in question for this species, since no actual observations have been extended at all to the course of meiosis.

26) *Plagusia dentipes* DE HAAN

The present species is a reddish crab with a carapace about 50 m. m. in breadth. It is commonly found along rocky shores of the southern parts of the Pacific coast of Japan.

It belongs to the Subfamily Plagusiinae of the Family Grapsidae, Oxyrhyncha, Brachygnata, Brachyura, Reptanita of the Order Decapoda. The crabs which furnished the material for the present work were collected from shores near the Mistui Institute of Marine Biology, at Suzaki near Shimode, Izu Peninsula at various times during October 1935 and March 1936.

The spermatogonia of the present species do not undergo a simultaneous division, but dividng cells are encountered intermingled with the resting cells. At metaphase, the chromosomes constitute a nearly round equatorial plate. The early and late spermatogonia can be differentiated according to their size. In Figs. 130 to 132, only early spermatogonia are shown. After careful counting in many equatorial plates of spermatogonia it is ascertained without doubt that the diploid number of chromosomes is 106. The chromosomes of the spermatogonial garniture show isomorphic nature; all chromosomes uniformly have acrocentric fibre attachment and vary in length from rods to spherules. It seems impossible to identify the homologous pairs, because of the considerably large number of chromosomes with only slight difference in size or shape.

As the division of the primary spermatocytes takes place synchronously, at least in those contained in one cyst, it is not difficult to find the dividing figures of cells, in which the chromosomes are seen in favorable condition for study, in a considerable number. Viewed from a pole of division it seems very clear that a metaphase plate is made up of 52 bivalent chromosomes which distribute themselves evenly throughout the whole area of the plate (Figs. 133–136). Size differences are noted among them in a rather remarkable degree, and the larger ones at least acquire a median transverse suture similar to the cases previously observed in different kinds of Decapoda; e.g. *Paralithodes camtschatica*, *Cambaroides japonicus*, etc. In this species, the two particular chromosomes, X and Y, are to be discovered also at the two sides of the metaphase plate holding a definite distance from the latter as was observed also in the preceding five species of the Family Grapsidae. They take likewise the central position in the metaphase plate and show a marked difference in their relative size. The smaller one is approximately 2/3 as long as the larger one, and this ratio seems to be definite without variation. Sometimes a constriction is observable at the middle portion of the larger one, the X. At the confronting ends they become gradually tapered and more or less pointed at the tips, while the opposite ends are rounded. The magnitude of the Y is comparable to those of tenth or eleventh grade larger than the very smallest size in the spermatogonial garniture. It is of particular importance that the X and the Y chromosomes are found constant in distance from each other from the plate in every cell of the same dividing phases (Figs. 137–140). The X and the Y seem to establish distance pairing during the diakinetic stage. The distance between the X and the Y chromosomes is approximately the same as those of *Gaetice depressus*. At anaphase, the X and the Y separate and migrate of the respective poles of the spindle with daughter halves of the autosomes. Thus it is evident that the chromosome constitution of the primary spermatocyte is represented by 52+XY=53; this number actually does represent

the haploid number of the present species.

The equatorial plate of the secondary spermatocyte is remarkably reduced in dimensions as well as the chromosomes themselves, compared with those of the primary spermatocyte although they have a strong resemblance in appearance with the latter. In the polar view of the equatorial plate, the chromosomes of dyad structure always count 53 without difficulty (Figs. 141–144). Theoretically speaking, there should be present two kinds of secondary spermatocytes of which one contains the X chromosome and the other involves the Y. But their distinction is practically impossible, for there are considerably many chromosomes with slight difference in dimensions which must include the X and the Y chromosomes.

The conclusion may be drawn that the diploid number of the present species is 106 (all rod), and the haploid number is $53=52+XY$. The present species is also remarkable for the presence of XY-type sex-mechanism in a distance pairing, as occurred also in five species of the Family Grapsidae.

ORDER ISOPODA

27) *Megaligia exotica* (*ROUX*)

The present species belongs to the Family Ligiidae, Oniscoidea of the Order Isopoda. It is one of the very common sea-side isopods found all around Japan. Body length of the species is 30–45 m.m. Specimens captured on rocky sea-side places in the vicinity of Oshoro near Otaru, in June, 1954, provided the material for study.

The testicular lobes containing spermatogonial cells are meagre. Since the division of spermatogonia was not take place simultaneously, the metaphase equatorial plates were very few in number. The diploid number 72 was decided through the observations of several reliable spermatogonial metaphase plates. As seen in Fig. 145, the spermatogonial garniture is of polymorphic nature. There are six pairs of metacentric V-shaped chromosomes and 30 pairs of acrocentric chromosomes of rod- and dot-like form in the spermatogonial complex.

The dividing figures of the primary and secondary spermtocytes were abundant in a certain testicular lobe. Every equatorial plate of the primary spermatocytes shows 36 bivalents which scatter equidistantly from one another throughout the whole area of the plate (Figs. 146–147). Size differences are noted among them in a rather remarkable degree; a few larger ones exhibit dumbbell shape, while the remaining smaller ones are rectangular in shape.

The secondary spermatocyte contains at metaphase 98 chromosomes of dyad nature, being much reduced in size as compared with primary spermataocyte (Fig. 148). Several comparatively larger dyads are noticed in the complex : they surely correspond to the V-shaped chromosomes observed in the spermatogonial complex. All the elements divide synchronously at anaphase of both first and second divisions, without showing any element of unusual behaviour.

The chromosome number of the present species, is, therefore, 72 (12V'+60R') in diploid and 36 in haploid in the male.

28) *Tylos granulata* MIERS

The present species, together with the preceding one, *Megaligia exotica*, is a member of the Suborder Oniscoidea, but differ in the family. It belongs to the Family Tyliidae, Oniscoidea of the Order Isopoda. It is a terrestrial isopod, being 10 m.m. in body length. It abounds in sandy sea-shores throughout Japan. The present study was based on the testicular material derived from several specimens captured in Ranshima, near Otaru in June, 1950.

A considerable number of dividing figures of spermatogonia could be observed among the spermatogonial cells in the resting stage in one testicular lobe. In the polar view, the chromosomes constitute a fairly rosette shaped equatorial plate, showing the larger chromosomes in the peripheral portion surrounding the smaller ones in central portion. As seen in Fig. 149, the spermatogonial garniture is polymorphic in nature. It was found after careful examination of several spermatogonial metaphases that 68 chromosomes constitute the male diploid complex of this species. Of these 68 chromosomes, 10 pairs are metacentric V-shaped elements and the remaining 24 pairs are acrocentric ones diminishing in size from long rod-shape to minute spherules.

Both first and second divisions of the spermatocytes occur simultaneously and abundantly in another testicular lobe. The chromosomes of the primary spermatocytes are observed with absolute clearness in the metaphase polar view, as they scatter evenly well apart from each other (Figs. 150–151). Every metaphase plate showed 34 bivalents with indisputable clearness. Size differences are rather remarkable: several larger bivalents show the form of a dumbbell with a transverse suture in middle region of each, while the remaining bivalents appear rectangular in form.

In the second metaphase the individual chromosomes exhibit a distinct dual nature; they are scattered all over the equatorial plate without any overlapping. Every metaphase plate examined shows 34 dyads without exception (Fig. 152). One dyad among them is metacentric in nature appearing as x shape. This dyad certainly corresponds to the largest V-shaped chromosome found in the spermatogonial garniture. No chromosome, peculiar in shape or behaviour, was found in either of the meiotic divisions.

To conclude, it is evident that the chromosome number of the present species is 68 in diploid and 34 in haploid in the male, and that the diploid complex is formulated as 20V'+48R'.

29) *Cleantiella isops* (GRUBE)

The present species is a member of the Family Idoteidae, Valvifera of the Order Isopoda. It is distributed widely along the coasts throughout Japan. It measures 20 to 30 m.m. in body length. The specimens obtained in the vicinity of the Toyoura Marine Biological Station, near Hakodate, in August, 1957, furnished the material for study.

Three pairs of testicular lobes of a testis do not show the same stage in process of maturation. The anterior lobe contains spermatogonial cells, the middle lobe is provided with spermatocyte of different stages, while the posterior lobe is filled with mature spermatozoa.

As occurred in the case of Decapoda, in the present species of Isopoda the spermatogonia do not undergo simultaneous division in a cyst, but dividing figures are encountered independently scattered among the cells in the resting stage. Several countings were made of the chromosomes in dividing spermatogonia. Every metaphase plate constantly showed 64 chromosomes as diploid number. An example is shown in Fig. 153. As seen in the figure, the spermatogonial garniture is polymorphic in nature; of 64 chromosomes, 34 elements are metacentric V-shaped and the remaining 30 are acrocentric rod- and dot-shaped elements. All the elements can be paired in the diploid complex.

Division of the spermatocyte took place in almost all the cells in a testicular lobe excepting cells at both terminal portions of the lobe which are in the stages of meiotic prophase. Many dividing figures, therefore, were observed in the primary and secondary spermatocytes; the bivalents take their appearance with distinct clearness, scattering evenly on the equatorial plate, as drawn in Fig. 199. In the present stage, therefore, the number of chromosomes can be counted with much ease and without error. There are observable 32 bivalents in every metaphase plate of the primary spermatocyte. About a half of the number of bivalents take the form of criss-cross tetrad or its modified forms. It is certain that these bivalents are the descendants of 17 pairs of V-shaped chromosomes in the spermatogonium. The remaining bivalents exhibit dumbbell and rectangular forms. Even in larger dumbbell shaped bivalents the transverse suture at the middle region could not be seen.

The metaphase polar view of the secondary spermatocyte shows 32 chromosomes of dyad nature (Fig. 155). All the the chromosomes corresponding to those of the spermatogonium are to be pointed out too in the garniture of the secondary spermatocyte : 17 metacentric V-shaped chromosomes and the remaining acrocentric ones varying from rod to dot shape.

The chromosome number of the present species is therefore 64 (34V'+30R') in diploid and 32 in haploid. There are no chromosomes which show form and behaviour characteristic to the sex-chromosomes during two meiotic divisions.

30) Idotea japonica RICHARDSON

The present species is also a member of the Family Idoteidae, Valvifera, of the Order Isopoda : individuals are 25–45 m.m. in body length. They are very common sea-shore animals in northern parts of Japan. The specimens for the present study were collected abundantly in the same place and simultaneously with the preceding species, *Cleantiella isops*.

The constitution and condition of the testicular lobes of the present species, are quite similar to those of the preceding species. The dividing figures of spermatogonia are found

here and there among the spermatogonial cells in the resting stage independently of the neighbouring cells. In the polar view the chromosomes assume a rosette form in their metaphase arrangement, as the larger ones occupy the peripheral position in the equatorial plate surrounding the smaller ones in the central part. In order to determine the number and form of chromosomes, many equatorial plates were examined in their polar aspect. Examples are given in Fig. 156. The diploid number observed in the spermatogonium was decided as 64. As is recognizable in the figure, the spermatogonial garniture is of polymorphic structure;of the 64 chromosomes, 30 are metacentric elements while the remaining 34 are all acrocentric varying in shape from long rod to short rod. According to their sizes and shapes all the chromosomes can be arranged into 32 homologous pairs. One pair amongst the metacentric chromosomes are satelite-like in structure, since each chromosome consists of a small dot-like body and a long rod-like one connected thereto by means of a fine thread. The other metacentric elements forming the 14 pairs are of V-shape. They all have nearly submedian attachment of the spindle fibres.

One pair of the three pairs of testicular lobes is filled with cells of primary and secondary spermotocytes in process of division. It is not difficult to find the dividing figures of spermatocytes, in which the chromosomes are seen in favourable condition for study. Viewed from the pole of division of primary spermatocytes, it is very clear that every metaphase plate contains 32 bivalent chromosomes, distributed evenly throughout the whole area of the plate (Fig. 157). All the bivalents take rectangular form indicating no sign of constitutional morphology. At anaphsae all the bivalents separate synchronously into two equal halves.

Careful observations of a considerable number of the metaphase plates of the secondary spermatocytes have revealed that the primary spermatocyte division actually gives rise to only one kind of secondary spermatocytes with respect to the complex of chromosomes. The chromosomes are generally much more slender in form, compared with that of the primary spermatocyte. Careful counting shows, without any exception, 32 dyads in every metaphase equatorial plate under study. Fig. 158 is an illustration of them. As seen in the figure, they all exhibit dyad nature. There are several dyads which evidently show by their shape that they are the descendants of V-shaped chromosome found in spermatogonial garniture. No chromosome having any peculiar form and behaviour could be detected throughout the two reduction divisions.

In summary, the chromosome number of the present species is 64 (30V'+34R') in diploid and 32 in haploid. The species is identical in chromosome number to the previously described one, *Cleantiella isops*, but is different in chromosome constitution; namely, the previous species has four more metacentric chromosomes and four less acrocentric ones than the present one. The presence of a pair of satellite-like chromosomes is also a characteristic feature peculiar to the present species.

31) Cymodoce japonicus RICHARDSON

The present species belongs to the Subfamily Sphaerominae, Sphaeromidae, Flabellifera of the Order Isopoda. It is 10–25 m.m. in body length. It dwells in the rocky sea-shore along the coasts of Japan from Hokkaido to Kyûsyû. The specimens for this study were collected from holes in wooden piers made by the shipworm, *Teredo*, along the sea-shore of the Akkeshi Marine Biological Station, in September, 1944.

The testes of the present species, consisting of three pairs of testicular lobes, are similar in structure with those of the previously described four isopods. The condition of each testicular lobes which show different stages of meiotic process in each, is also quite comparable to that of the preceding four isopods, *Megaligia exotica*, *Tylos granulata*, *Cleantiella isops* and *Idotea japonicus*.

Dividing figures of spermatogonia were observed abundantly within a lobe. The chromosome arrange themselves well apart from one another, forming a fairly circular equatorial plate. Careful counting of excellent spermatogonial metaphase plates gave the diploid number of 58 without even slight doubt. As seen in Fig. 159, the spermatogonial garniture is of polymorphic nature: of 58 chromosomes, 32 are metacentric elements of V-shape and 26 are acrocentric ones of rod- or dot-shape. All the chromosomes of the spermatogonium can be arranged into to 29 homologous pairs.

In the metaphase polar view of the primary spermatocyte the bivalents take their appearance with distinct clearness, scattered evenly on the equatorial plate, as drawn in Figs. 160 and 161. In the present stage, therefore, the number of chromosomes can be counted with much ease and without mistake. The primary spermatocyte contains 29 bivalents without exception. There are several bivalents which are considered to be ring-tetrads. They evidently correspond to the larger metacentric V-shaped chromosomes in the spermatogonium. The remaining bivalents exhibit a dumbbell or rectangular shape. No transverse suture across the middle region of each dumbbell-shaped bivalent, though it is rather common in the bivalents of Decapoda, could be seen in the present species. At anaphase, all the bivalents separate into two equal halves and migrate synchronously to the poles of the spindle. No chromosome, peculiar in shape and behaviour, was found in the present division.

As obvious from the mode of division of the first spermatocyte, there is only one kind of secondary spermatocytes concerned in the chromosome complex. In the polar view of the second spermatocyte at metaphase, 29 chromosomes of dyad structure constitute a fairly circular equatorial plate (Fig. 162). Among these dyads, several appear as metacentric ones. They may be the descendants of larger V-shaped chromosomes in the spermatogonial garniture. At anaphase, nothing particular is found in the mode of the secondary spermatocyte division; every dyad divides into two equal daughter monads.

It may then be summarized that the chromosome number of the present species is 58 in diploid and 29 in haploid in the male, and that the diploid complex is represented by 32V'+26R' As described above, the present species throughout the two reduction divisions

contains no particular chromosome regarded as the sex-determining element.

32) *Tecticeps japonicus* IWASA

The present species is one of the marine isopods belonging to the Subfamily Sphaerominae, Sphaeromidae, Flabellifera of the Order Isopoda. It measures 10–15 m.m. in body length. They are common around the sea-shores of the Hokkaido and the Kurile Islands. The material upon which the present study was based was obtained from animals collected in the vicinity of the Akkeshi Marine Biological Station, Hokkaido in June and July in the years, 1950 to 1954.

The testes of the present species consist of three pairs of testicular lobes, each having a spindle shape, as in the preceding five species. The terminal parts of the testicular lobes are occupied by spermatogonial cells and the other parts are filled with cells at various stages of meiosis. The dividing figures of spermatogonial cells are not frequent, by found the metaphase figures being rather rare. Examples of ideal metaphase plates are shown in Fig. 163 and 164. After careful counting of the chromosomes in several such plates, it was found that the diploid number was invariably 63. It is quite natural to expect the existence of a single X-chromosome based on the occurrence of the odd chromosome number in the spermatogonial division. The spermatogonial chromosomes are polymorphic in the complex; the complex consists of a certain number of acrocentric and metacentric chromosomes. As seen in Fig. 165 which is a serial alignment of the supposed pairs of chromosomes, there are 18 pairs of acrocentric chromosomes of rod-type, 13 pairs of metacentric of V- and J-shape, and a single, large metacentric element. The latter is very conspicuous among the others since it is the largest of all in size, submedian in fibre attachment, and unpaired having no homologous mate. This finding is explicable only by assuming this element to be the X-chromosome.

The majority of germ cells are found in process of meiosis in the testicular lobes fixed in June and July. The first and second divisions were available for study in the material collected in approximately the first week of July.

The primary spermatocytes in the metaphase side view clearly and invariably show remarkable precocious migration of a V-shaped chromosome of outstandingly large size, in striking contrast to the autosomal bivalents which lie in the equatorial plate. This element which makes the precocious migration to one of the poles is in all probability the X-chromosome because of its characteristic feature as seen in the former division and of its particular behaviour like the X of many animals (Figs. 170–171). In the early anaphase stage (Fig. 172) this particular element reaches one of the poles, while the autosomal elements are on the way thereto. Apparently it is univalent in structure.

The number of bivalents observed in the equatorial plate is 31 without exception. In addition to these autosomal bivalents, the X-chromosome makes its appearance when the focus of the microscope is changed a little upward or downward from the equatorial plate, being very conspicuous for its large V-shaped, univalent configuration (Figs. 167–

168). The majority of the bivalents take the form of a criss-cross tetrad, while the remaining ones are dumbbell and rectangular in form. These bivalents in criss-cross shape are surely the descendants of the metacentric V-shaped chromosomes in the spermatogonial garniture. In the first meiotic division the X-element moves to one of the poles without division, resulting in the production of two kinds of secondary spermatocytes; one having the X and the other containing no such one.

Observations of the diakinetic nucleus revealed that one large element has remained in a heteropycnotic condensation showing a distinct V-shape. This is no other than the X-element (Fig. 166) which corresponds to such an element observed in the first meiotic division.

It is thus evident that the X-chromosome of this form is characterized by a distinguishable V-shape and large size, remarkable precocious separation in the first meiotic division and heteropycnosis in the growing stage.

The chromosome constitution of the primary spermatocyte is shown by the formula, $32=31+X$, as the haploid number of the present species in the male.

As the result of the first division, it is evident that there are to be produced two kinds of secondary spermatocytes, the X-class cell containing the X-element and no-X-class cell. The present material furnished a few dividing secondary spermatocytes in which only an X-class cell was observed (Fig. 169). The equatorial plate of the secondary spermatocyte is remarkably reduced in dimensions as well as the chromosomes themeselves compared with the size of the primary spermatocyte, although they have strong resemblance in appearance to the latter. The equatorial plate contains 31 dyads and a large V-shaped chromosome in addition which is the X-element.

On the basis of the above facts, it is concluded that the present species shows male heterogamety represented by an X-O mechanism. This finding is quite unique in the Order Isopoda, so far as the chromosomal survey has gone.

ORDER AMPHIPODA

33) Anisogammarus annandalei (TATTERSALL)

The present species is a common one of fresh water amphipods in Hokkaido. It belongs to the Family Gammaridae, Gammaridea of the Order Amphipoda. It measures 15 to 20 m.m. in body length. The specimens from which the material was derived were collected in July 1934 in a small stream running through the Hokkaido University campus.

Since the divisions of the spermatogonia are not simultaneous in the testicular cyst, the metaphase figures are not abundant. After careful counting of the chromosomes, the diploid number was determined to be constantly 54 in the spermatogonial complex; all the elements are uniformely acrocentric in their fibre attachment. They range from rods of medium size to those dot-like in shape. It is, therefore, impossible to pair them by a

comparison of their size and shape. The even number of chromosomes suggests the occurrence of an XY-pair. Careful observations reveals the existence of a chromosome which is characterized by its small size. It is solitary having no homologous mate in the complex and is about two-thirds the size of the elements of the smallest pair of chromosomes. Because of its unpaired condition and of its smallest size, this element is probably the Y chromosome (*y* in Figs. 173–174). It is difficult to identify the X chromosome in the diploid complex.

The resting nucleus of the primary spermatocyte contains two very conspicuous chromatin nucleoli (Figs. 179–186, *xy*). They are easily distinguished from the plasmosome nucleolus by the intense affinity for haematoxylin and by the peculiar form as well. Further, one of them is smaller, having a size one-third that of the other. Through all stages of the growth period, these two nucleoli are connected with each other by a fine chromatin thread. In the leptotene stage, the chromatin thread is considerably elongated, having two nucleoli at each end (Figs. 179–182). The association between the plasmosome and the chromatin nucleoli does not occurr through the growth period. At the commencement of diakinesis, the chromatin thread becomes shortened and the larger nucleolus assumes an angular shape (Figs. 183–186). Under this condition, the difference between the two elements of the nucleoli becomes very evident.

At metaphase of the first division, these two elements forming the nucleoli, lie in the equatorial plate assuming an end-to-end conjugation with their long axes at right angles to the plate (Fig. 187). At anaphase, they separate and migrate to the opposite poles. Judging from such a behaviour, these two unequal elements seem to be no other than the sex-chromosome of the present species, the larger being the X-chromosome and the smaller the Y-element.

In the polar view of the first division, 27 chromosome are arranged with distinct clearness, as shown in Figs. 175–178. In the side-view (Fig. 187), the XY complex is clearly distinguishable from the autosomal bivalents. It is evident from the above facts that the chromosome formula of the primary spermtocyte is $26+XY=27$.

Every equatorial plate of the secondary spermatocytes shows 27 chromosomes (Figs. 188–191). There are two kinds of secondary spermatocytes, of which one contains the X-chromosome and the other the Y. But the distinction of them is practically impossible, because of the scantiness of distinguishing characters available for identification.

As mentioned above, the present observations establish the existence of the XY-mechanism of sex-chromosomes in male germ cells of this species. The diploid number is 54 and the haploid number is 27.

V. PART II. CHROMOSOME MORPHOLOGY IN RELATION TO TAXONOMICAL CLASSIFICATION OF THE CRUSTACEA

The taxonomy, variation and distribution of animals have been dealt with by a number of taxonomists. On the other hand, the chromosome cytology has largely contributed to animal systematics in several different ways: cytological data serve as useful and fundamental criteria for the diagnosis of species, and for the understanding of the evolutionary mechanism of organisms, since changes in chromosomes involving various types of inner structural rearrangements play an important role in the formation of species (White '57 a,b). The application of cytological methods to taxonomy has increasingly made possible the solution of discrepant taxonomical matters that are always difficult for non-cytologists to solve. The cytotaxonomic differences which are generally noted by cytologists deal with differences in chromosome number, or in the sizes and shapes of some of the chromosomes in the complex.

In the following pages, the author shall refer to any cytologically detectable differences in related orders, families, genera and species of the Crustacea. Further, consideration will be given to the contribution of the cytology to the taxonomy of some crustacians, briefly viewing the evolution of three orders, Decapoda, Isopoda and Amphipoda of the Class Crustancea.

Table 1 shows the numerical relationship of the chromosomes in the Decapoda, Isopoda and Amphipoda of the Crustacea so far reported on.

It is evident by reference to Table 1 that, in the Decapoda, the chromosome number shows a wide range of variation from $2n=82$ in *Gebia major* (Oka '41) to $2n=254$ in *Eupagurus ochotensis* (Niiyama '44). The Isopoda give rather small counts which vary from $2n=12$ in *Anilocra mediterranea* (Callan '40) to $2n=72$ in *Megaligia exotica* (Niiyama '59). In the Amphipoda, on the other hand, the counts are comparatively uniform giving from $n=23$ in two species of *Maera* to $n=27$ in *Gammarus duebeni* (Le Calvez et Certain '51), excepting one species, *Marinogammarus pirloti* (Orian and Callan '57) which shows a chromosomal polymorphism such as $n=29$, 30, 31, 32.

1) Order Decapoda

The chromesome number of the Decapoda shows a considerable variation within the Order (see Table 1). In the Suborder Natantia chromosome counts have been made in only three species, *Crangon cataphractus* (Carnoy '85), *Pandalus borealis* (Leopoldseder '34) and *Penaeus japonicus* (Niiyama '48). The data from the former two species may be doubtful by present standards of cytology, since the chromosome figures presented in their papers seem to be derived from poorly preserved specimens. The counts, $2n=92$, $n=46$, in *Penaeus japonicus* is a rather small number among the Decapoda and seems to have closer relationship to the. Brachyura than to the other groups of Reptantia of the Decapoda. Definite conclusion, however, must be postponed because there is available only one

Table 1. Chromosome numbers of decapods, isopods and amphipods so far reported

DECAPODA

Species	2n	n	Author
Suborder Natantia			
Group Penaeidea			
Family Penaeidae			
Penaeus japonicus	92s (14V′+78R′)	46 ♂	Niiyama '48
Group Eucyphidea			
Tribe Pandaloidea			
Family Pandalidae			
**Pandalus borealis*		34 ♂ I 32+2X ♂ II	Leopoldseder '34
Tribe Crangonida			
Family Crangonidae			
**Crangon cataphractus*		40–44 ♂	Carnoy '85
Suborder Reptantia			
Group Palinura			
Family Palinuridae			
Panulirus japonicus	140 (12V′+128R′)	70 ♂	Niiyama '36a,b
Group Astacura			
Family Potamobiidae			
**Astacus fluviatilis*		ca. 58 ♂	Prowazek '02
Cambarus virilis	200s	100 ♂	Fasten '14
Cambarus immunis (?)		98+8 ♂ I	Fasten '14
Cambarus clarkii	192s (6V′+186R′)	96 ♂	Niiyama '34
Cambaroides japonicus	196s (all rod)	98 ♂	Niiyama '34
Family Nephropsidae			
**Homarus*		18 ♂	Labbé '40
Nephrops japonicus	164s (all rod)	82 ♂	Niiyama '39
Nephropsis carpenteri	152s (30V′+122R′)	76 ♂	Niiyama '39
Group Anomura			
Tribe Thalassinidea			
Family Callianassidae			
Gebia major	82s,o	41 ♂	Oka '41
Tribe Galateidea			
Family Galateidae			
Cervimunida princeps	109s (18V′+91R′)	53+XXY ♂ I	Niiyama '59
Tribe Hippidea			
Family Hippidae			
Hippa talpoides		60 ♂	Nichols '09
Tribe Paguridea			
Family Paguridae			
Eupagurus ochotensis	254s (18V′+236R′)	127 ♂	Niiyama '44
**Eupagurus prideuxii*		12 ♀	Weismann & Ishikawa '88
Family Coenobitidae			
Coenobita rugosa	230s (all rod)		Niiyama This paper
Family Lithodidae			
Paralithodes camtschatica	208s (all ord)	104 ♂	Niiyama '36a,b
Paralithodes platypus	206s (18V′+188R′)	103 ♂	Niiyama This paper

Group Brachyura			
Tribe Oxystomata			
Family Carapidae			
Matuta lunaris	94s (4V′+90R′)	47 ♂	Niiyama '42
Family Leucosiidae			
Philyra pisum	114s (4V′+110R′)	57 ♂	Niiyama This paper
Family Raninidae			
Ranina ranina	106s (24V′+82R′)	53 ♂	Niiyama '42
Tribe Brachygnata			
Superfamily Oxyrhyncha			
Family Majidae			
Macrocheira kaempferi	106s (16V′+90R′)	53 ♂	Niiyama '39
Superfamily Brachyrhyncha			
Family Portunidae			
Subfamily Portuninae			
Ovalipes punctatus	103s (4V′+99R′)	51+X ♂ I	Niiyama '41
Subfamily Carcininae			
**Carcinus maenus*		30–40 ♂	Carnoy '85
Subfamily Lupinae			
Scylla serrata	106s (all rod)	53 ♂	Niiyama '42
Family Atelecyclidae			
Telmessus cheiragonus	124s (18V′+116R′)	62 ♂	Niiyama '42
Family Cancridae			
Cancer gracilis		52 ♂	Fasten '24
Cancer magister		60 ♂	Fasten '18
Cancer oregonensis		56 ♂	Fasten '24
Cancer products		58 ♂	Fasten '24
Family Xanthidae			
Atergatis floridus	104s (4V′+100R′)	52 ♂	Niiyama This paper
Lophopanopeus bellus		62 ♂	Fasten '26
**Menippe mercenaria*	51–80s	25–28 ♀	Binford '13
Family Potamonidae			
Potamon dehaani	82s (about half V′ or J′)	41 ♂	Yanagita '44
**Telphusa fluviatilis*	78s	38+2X ♂ II	Delpino '34
Family Grapsidae			
Subfamily Grapsinae			
Pachygrapsus crassipes	118s (all rod)	58+XY ♂ I	Niiyama This paper
Subfamily Varuninae			
Hemigrapsus sanguineus	128s (all rod)	63+XY ♂ I	Niiyama '38
Hemigrapsus penicillatus	138s (2V′+136R′)	68+XY ♂ I	Niiyama This Paper
Eriocheir japonicus	148s (all rod)	73+XY ♂ I	Niiyama '37
Gaetice depressus	152s (12V′+140R′)	75+XY ♂ I	Niiyama This paper
Subfamily Plagusiinae			
Plagusia dentipes	106s (all rod)	52+XY ♂ I	Niiyama '37a,b

ISOPODA

Suborder Asellota			
Family Asellidae			
**Asellus aquaticus*	20–30s	8–10 ♂	Carnoy '85
Asellus aquaticus	16s	8 ♂	Vandel '38 '41
Asellus aquaticus	16s	8 ♂ I	Vitagliano '47
**Asellus aquaticus*	17s	7+X ♂ I	Dworak '35

Asellus (*Proasellus*) *meridianus*	16s	8 ♂ I	Vandel '38. '41
Asellus nipponensis	14s	7 ♂ I	Sugiyama '33
Stenasellus virei		27 ♂ I	Vandel '41, '47
Family Janiridae			
Jaera marina forsmani		8+XYY ♀ I	Steiger & Bocquet '54
Jaera marina ischiosetosa		12+XYY ♀ I	Steiger & Bocquet '54
Suborder Oniscoidea			
Family Oniscidae			
Subfamily Armadillidiinae			
Armadillidium opacum	54s	27 ♂	Radu '30
Armadillidium nasatum		28 ♀ I	Vandel '41
Armadillidium vulgare	54s	27 ♂ I	Radu '31
Armadillidium vulgare		28 ♂ I	Vandel '41
Subfamily Oniscinae			
Oniscus asellus	32s	16 ♂ ♀	Nichols '01, '02' '04
Chaetophiloscia elongata		24 ♀ I	Vandel '41
Philoscia gallicus		24 ♀ I	Vandel '41
Subfamily Porcellioninae			
Porcellio scaber	56s (all V')	28 ♂	Imai & Makino '40
Porcellio scaber		28 ♀ I	Vandel '41
Porcellio gallicus		28 ♀ I	Vandel '41
Porcellio loevis		28 ♀ I	Vandel '41
Porcellio muscorum		24 ♀ I	Vandel '41
Family Ligiidae			
Ligia italica		28 ♂ ♀	Mir '39
Ligia oceanica		30 ♂	Mir '39
Ligidium hypnorum		31 ♂	Mir '39
Megaligia exotica	72s (12V'+60R')	36 ♂	Niiyama '59
Family Tyliidae			
Tylos granulata	68s (20V'+48R')	34 ♂	Niiyama '59
Suborder Valvifera			
Family Idoteidae			
Cleantiella isops	64s (34V'+30R')	32 ♂	Niiyama '59
Idotea irrorata		28 ♂	Nichols '09
Idotea japonica	64s (30V'+34R')	32 ♂	Niiyama '59
Suborder Flabellifera			
Family Cymothoidae			
Anilocra mediterranea	12s, o	6 ♂	Callan '40
Family Sphaeromidae			
Cymodoce japonica	58s (32V'+26R')	29 ♂	Niiyama '59
Tecticeps japonicus	63s (27V'+36R')	31+X ♂ I	Niiyama '56
Suborder Epicaridea			
Family Bopyridae			
*Epipenaeon japonica	16cl	8p. b.	Hiraiwa '36

AMPHIPODA

Suborder Gammaridea			
Family Gammaridae			
Anisogammarus annandalei	54s (all rod)	26+XY ♂ I	Niiyama '35 '50
Carinogammarus roeseli		26 ♂ I	Le Calvez '49
Echinogammarus berilloni		26 ♂ I	Le Calvez '49
**Gammarus chevreuxi*		ca. 22	Huxley '23
**Gammarus chevreuxi*	26s	12+XY ♂ I	Palmer '25, '26
Gammarus chevreuxi	52s	26 ♂ I	Le Calvez et Certain '51
Gammarus chevreuxi		26 ♀ II	Orian & Callan'57

**Gammarus duebeni*		13	Le Roux '33
Gammarus duebeni	54s (over 50V')	27 ♂ I	Le Calvez et Certain '51
Gammarus duebeni		27 ♀ II	Orian & Callan '57
Gammarus locusta		26 ♂ I	Poisson et Le Calvez '48
Gammarus locusta		26 ♀ II	Orian & Callan '57
Gammarus marinus	52s	26 ♂ I	Le Calvez et Certain '51
Gammarus pulex pulex	52s	26 ♂ I	Poisson et Le Calvez '51
Gammarus pulex	52, 53cl	26, 27 ♀ II	Orian & Callan '57
Gammarus pungens	48s	24 ♂ I	Le Calvez et Certain '51
Gammarus zaddachi		26 ♂ I	Le Calvez '49
Maera othonis		23 ♂ I	Le Calvez et Certain '51
Maera grossimana		23 ♂ I	Le Calvez et Certain '51
Marinogammarus finmarchus		26 ♀ II	Orian & Callan '57
Marinogammarus longipes		26 ♂ I	Le Calvez '49
Marinogammarus marinus		25, 26 ♀ II	Orian & Callan '57
Marinogammarus obtusatus		26 ♀ II	Orian & Callan '57
Marinogammarus pirloti		29, 30, 31, 32 ♀ II	Orian & Callan '57
Niphargus plateaui var. *elongatus*		25 ♂ I	Le Calvez et Certain '51
Niphargus tatrentis var. *lunzensis*	50s (2V'+48R')	25 ♂ I	Le Calvez et Certain '51
Family Talitridae			
Orchestia gammarella		25 ♂ I	Poisson et Le Calvez '48
Orchestia mediterranea		25 ♂ I	Le Calvez et Certain '51
Talitrus saltator		25 ♂ I	Poisson et Le Calvez '48
Talorchestia deshyesei		25 ♂ I	Le Calvez et Certain '51
**Talorchestia longicornis*		18	Nichols '09

Abbreviations used :- s : spermatogonium ; o : oogonium ; cl : cleavage ; p.b. : polar body ; I : primary spermatocyte : II : secondary spermatocyte ; V' : metacentric chromosome ; R' : acrocentric chromosome; *The data marked with asterisks are those unsatisfactory by present-day-standards of cytology.

reliable datum at present.

The Suborder Reptantia is divided taxonomically into four groups, viz., Palinura, Astacura, Anomura and Brachyura. In the group Palinura, only one species, *Panulirus japonicus*, was studied cytologically by the present author (Niiyama '36a, b). It shows 140 in $2n$ and 70 in n. The characteristic feature is that this species has compound ring-tetrads together with the ordinary bivalents in the primary spermatocyte : they are the descendants of large V-shaped metacentric chromosomes in the spermatogonial cells. Such bivalents have never been reported to occur in other species of Crustacea so far studied. Accordingly, it is rather difficult to consider the relationship of this species to other groups of Crustacea.

In the Group Astacura, eight species belonging to two families, Potamobiidae and Nephropsidae, were studied cytologically. Setting aside the two suspicious cases of *Astacus fluviatilis* $n=ca.$ 58 (Prowazek '02) and *Homarus* $n=18$ (Labbé '04), four members

of the Potamobiidae have more or less than 200 chromosomes, while two members of the Nephropsidae are characterized by a smaller number than the former. The Astacura seems to have a close relationship to the Tribe Paguridea of the group Anomura in view of from the chromosome set-up. Two species of the Family Nephropsidae, *Nephrops japonicus* and *Nephropsis carpenteri*, seem to be closely related from the view point of chromosome morphology on the basis of the presence of unusual quadripartite bivalents in each species. Special interest is attracted from the morphological view-point when one considers the evolution of the chromosome complex in Crustacea that in spite of their close taxonomical relation, the former species has 164 chromosomes of rod-shape, while the diploid complex of the latter consists of 30 V-shaped metacentric chromosomes and 122 rod-shaped ones.

In the Group Anomura, the chromosome number varies considerably among the different Tribes. The group is divided into four Tribes: Thalassinidea, Galateidea, Hippidea and Paguridea. In the former three Tribes, the following chromosome numbers have been reported: $2n=82$ in *Gebia major* (Oka '41), $2n=109$ in *Cervimunida princeps* (Niiyama '59) and $n=60$ in *Hippa talpoides* (Nichols '09). Those counts fairly approximate those of the Brachyura Group. Moreover, that *Cervimunida princeps* has an X_1X_2Y-type sex-mechanism in the male, suggests the occurrence of kinship to the Brachyura in which several species have an XO or XY sex-mechanism in the male, since it has repeatedly been stated that the XX-Y mechanism was secondarily derived from either an XO- or XY-condition by translocation involving an autosome and sex-chromosomes (White '40, '41, '54, Cooper '46, '51, Hughes-Schrader '47, '50). In contrast to the above three Tribes, the Paguridea is characterized by a high number of chromosomes, all the species so far reported having over 200. The highest number in animals so far studied was obtained in *Eupagurus ochotensis* (Niiyama '44) which shows $2n=254$. With respect to the number of chromosomes, there is a close relationship between Paguridea and Astacura.

In the Group Brachyura, twenty-three species have been investigated cytologically. All the species have about 100 chromosomes, excepting several species of the Family Grapsidae in which there have hitherto been reported $2n=152$ in *Gaetice depressus* (Niiyama This apaper), $2n=148$ in *Eriocheir japonicus* (Niiyama '37), $2n=138$ *Hemigrapsus penicillatus* (Niiyama This paper), $2n=128$ in *Hemigrapsus sanguineus* (Niiyama '38), $2n=118$ in *Pachygrapsus crassipes* (Niiyama This paper) and $2n=106$ in *Plagusia dentipes* (Niiyama '37a,b). All these species have an XY sex-mechanism in a distance pairing. Considered from the chromosome number and the condition of sex-chromosomes, it seems probable that the Family Grapsidae is a group slightly separated from the other families of the Brachyura. The XO sex-mechanism which was found to occur in a species of the Family Portunidae is a unique example. Decision as to the relationship of this family to the others must be postponed until the observations on many species of this family have completed.

The Reptantia are in general divided into two classes on the basis of chromosome count.

One class is characterized by more or less than 200 chromosome, while the other by 100 or thereabouts. In dimensions the cells are larger in the former class than in the latter. There is, however, an exception in that *Ranina ranina* has large sized cells comparable to those of *Panulirus japonicus*, in spite of the fact that it belongs to the Brachyura with 106 chromosomes in diploid (Niiyama '42).

As is noted in the above discussion, the basic cytological data for understanding the systematic relationship in the Decapoda are very incomplete at present. Under the present status of cytological knowledge, it is very difficult to reach any definite conclusion to generalize the relationship between taxonomical classification and cytological features: a much more complete picture of cytotaxonomy may be expected after an extensive collection of cytological data in many forms of animals.

2) Order Isopoda

Reference to Table 1 indicates that, in the Order Isopoda, reports on the chromosomes of twenty-eight species have so far been published.

Vandel ('41), on the basis of his extensive studies, expressed the view that the haploid number of the marine isopods and of the related terrestrial and fresh-water species is 28 or thereabouts. According to him the huge chromosomal elements found in species of *Asellus* are to be regarded as having resulted from a fusion of several small chromosomes as found in *Stenasellus*, though there is no positive proof for that view. Surely, all the chromosomes of *Asellus aquaticus* are large metacentric ones. Vandel states that the chromosome numbers of the true Oniscoidea tend to increase with the evolution of the species: the following relationship seems to occur in the chromosome number of isopods.

Marine species (n, 28) ...... Asellus (n, 8) Trichoniscus ...... Oniscidae (n, 16 to 24) ...... Porcellio Armadillidium (n, 28)

The two extreme forms show a high chromosome number, while the intermediate species have a smaller number, which have probably resulted from fusion or association of several chromosomes.

Looking upon the data presented in Table 1, it becomes evident that among marine species, *Cleantiella isops*, $n=32$ (Niiyama '59), *Idotea japonicus*, $n=32$ (Niiyama '59), *Cymodoce japonicus*, $n=29$ (Niiyama '59) and *Tecticeps japonicus*, $n=31+Y$ (Niiyama '56), have high chromosome number, while two subspecies of *Jaera marina* with $n=8+XYY$ and $n=12+XYY$ (Steiger and Bocquet '54), show a number lower than 28. Certain terrestrial and half-terrestrial species, also, have a high number of chromosome: for instance, *Ligidium hypnorum*, $n=31$ (Mir '39), *Ligia oceanica*, $n=30$ (Mir'39), *Tylos granulata*, $n=34$ (Niiyama '59) and *Megaligia exotica*, $n=36$ (Niiyama '59) are available for reference. In the authors' opinion, the relationship between the chromosome number and the taxonomical situation in the isopod Crustacea is difficult to establish in the present status of investigation. Sufficient information needful for a decision can not be expected until

after the chromosomes of many species have been exhaustively investigated. The data on the chromosomes of isopod Crustacea are too scanty at present to justify a generalization as to the relationship between the chromosome number and taxonomical arrangement of the species. There is no positive evidence upon which to enter into a consideration of reduction in the chromosome number as a result of chromosome fusion.

3) Order Amphipoda

The data shown in Table 1 indicate that cytological investigations have been made on twenty-three species in the Amphipoda by several authors. The studied species are all restricted and belong to two families, Gammaridae and Talitridae of the Suborder Gammaridea. In order to formulate any rule for general application concerning the relationship between cytological data and taxonomical situation, it is necessary to accumulate additional accurate cytological data on many species covering many families of this order.

It is evident from the data in Table 1 that the haploid number of all the species of the Family Talitridae is $n=25$ in the male (Poisson et Le Calvez '48: Le Calvez et Certain '51), while in the Family Gammaridae, the fundamental number seems to be $n=26$. Almost all the species studied have 26 counts in haploid. Le Calvez ('51) stated that the haploid number, 25, occurring in two species of *Niphargus* is caused by the fusion of chromosomes, since one pair of large V-shaped metacentric chromosomes exists among the spermatogonial garniture (Le Calvez et Certain '51). Le Cavlez et Certain ('51) suggested with considerable justification that both Palmer's and Le Roux's determinations, a haploid number of 13 for *Gammarus chevreuxi* (Palmer '25, '26), and *Gammarus duebeni* (Le Roux '33), were based on inadequate technique and were erroneous. There are several species which deviate from the fundamental number (n, 26); viz., two species of *Maera* have $n=32$ (Le Calvez et Certain '51), *Gammarus pungens* has $n=24$ (Le Calvez et Certain '51) and *Gammarus duebeni* shows $n=27$ (Orian and Callan '57, Le Calvez et Certain '51). Le Calvez et Certain ('51) express the opinion that the reduced number is caused by disappearance of some chromosomes by their transformation into heterochromatin.

Orian and Callan ('57) reported evidence of chromosomal polymorphism to occur in three species of the Gammaridae in the second occyte showing that there occur n, 26 and 27 in *Gammarus pulex*, n, 25 and 26 in *Marinogammarus marinus*, and n, 29, 30, 31, and 32 in *Marinogammarus pirloti*. According to their view, there is no evidence to show that this polymorphism is in accord with Robertson's fragmentation-fusion' system, but it appears to be due to the occurrence of supernumerary chromosomes. To the present author's veiw, there is a question whether the number of chromosomes has been correctly counted in the second oocyte division in aceto-orcein squash preparations.

At the present status of investigation, it seems quite difficult to formulate any rule for general application as to the relationship between the chromosome number and taxonomical arrangement of species in these orders, since the basic data for understanding

Table 2. Sex-determining mechanism so far reported in the Crustacea

Species	2n	Sex-chrom.	Author
Phyllopoda			
Branchipus vernalis	23	X-O ♂	Baker & Rosof '27, '28a,b
Ostracoda			
Cyclocypris globosa		X-O ♂	Dietz '58
Cyclocypris laevis		X-Y ♂	Dietz '58
Cyclocypris ovum		$X_{-3-6}Y$ or $X_{-4-7}Y$ ♂	Dietz '58
Cypria exsculpta		X_{1-3}-Y or $X_{1-3}X_4$-Y ♂	Dietz '58
Cypria ophthalmica		$X_{1-3}X_4$-Y ♂	Dietz '58
Cypris compacta		X_{1-3}-Y ♂	Dietz '58
Cypris dietzi		X_{1-3}-Y ♂	Dietz '58
Cypris fodiens		X_{1-3}-Y ♂	Dietz '58
Cypris whitei		X_{1-3}-Y ♂	Dietz '58
Heterocypris incongruens		X_{1-6}-Y ♂	Bauer '40
Notodromas monacha		X_{1-2}-O ♂	Dietz '54
Physocypria kliei		X_{1-3}-O ♂	Dietz '58
Platycypris baueri		X_{1-3}-O ♂	Dietz '58
Scottia browniana		X_{1-3}-O ♂	Dietz '58
Copepoda			
Centropagis typicus		X-O ♂	Heberer '32
Cyclops affinis		*X-O ♀	Matschek '10
Cyclops fuscus var. *distinctus*		*X-O ♀	Matschek '10
Cyclops phaleratus	13	*X-O ♀	Braun '09
Cyclops phaleratus		*X-O ♀	Matschek '09, '10
Cyclops prasinus	11	*X-O ♀	Braun '09
Cyclops prasinus		*X-O ♀	Matschek '10
Cyclops serrulatus		*2X-O ♀	Matschek '10
Cyclops vernalis		*X-O ♀	Matschek '09 '10
Ectocyclops strenzki		X-O ♀	Beervann '54
Isopoda			
Asellus aquaticus	17	*X-O ♂	Dworak '35
Jaera marina forsmani		*X-Y_1Y_2 ♀	Steiger & Bocquet '54
Jaera marina ischiosetose		*X-Y_1Y_2 ♀	Steiger & Bocauet '54
Tecticeps japonicus	63	*X-O ♂	Niiyama '56
Amphipoda			
Anisogammarus annandalei	54	X-Y ♂	Niiyama '50
Gammarus chevreuxi	26	*X-Y ♂	Palmer '25, '26
Decapoda			
Cambarus immunis (?)		*8X-O ♂	Fasten '14
Cervimunida princeps	109	X_1X_2-Y ♂	Niiyama '59
Eriocheir japonicus	148	X-Y ♂	Niiyama '37
Gaetice depressus	152	X-Y ♂	Niiyama This paper
Hemigrapsus sanguineus	128	X-Y ♂	Niiyama '38
Hemigrapsuss penicillatus	138	X-Y ♂	Niiyama This paper
Ovalipes punctatus	103	X-O ♂	Niiyama '40
Pachygrapsus crassipes	118	X-Y ♂	Niiyama This paper
Pandalus borealis	34	*2X-O ♂	Leopoldseder '34
Plagusia dentipes	106	X-Y ♂	Niiyama '37 a, b
Telphusa fluviatilis	78	*2X-O ♂	Delpino '34

* The data regarding which there is some doubt are marked with asterisks. For reference, see Makino's list, 1956.

the matter are very incomplete. It is necessary to accumulate additional accurate cytological data on a very large scale in the Crustacea in general.

VI. PART III. SEX-DETERMINING MECHANISM IN THE CRUSTACEA

The vast majority of higher animals have separate sexes and possess genetic mechanism for their sex-determination. Genetic sex-determining mechanisms are of many different kinds. Extensive cytological investigations during more than fifty years since 1900 have presented the fact that there occur two major types in cytological mechanism of sex-determination in animals. The one is referred to as male heterogamety which is characterized by heterogametic sex-chromosomes occurring in the male, while the other deals with female heterogamety which involves a heterogametic condition existing in the female. Accurate data on male heterogamety have been ascertained from both genetical and cytological standpoints to occur in almost all the orders of the Insecta (Arthropoda), while clear cytological and genetical proof for female heterogamety in insects has been offered in two orders, Lepidoptera and Trichoptera. As given in the following, the sex-determining mechanism has been reported cytologically in many species of Crustacea covering six orders, as having either male or female heterogamety. The data are arranged in the form of a table (Table 2).

By reference to Table 2, it becomes evident that, except for certain questionable reports, the sex-determining mechanism in the Crustacea is of XO- or XY-type. In certain species of the Decapoda, Amphipoda and Phyllopoda, the XO- or XY mechanism was found to occur in the male (Baker and Rosof '27, '28a,b, Niiyama '37a, b, '38, '40, '50, '56). Very recently, an X_1X_2-Y sex-mechanism was reported on the basis of observations of male germ-cells of *Cervimunida princeps* by the present author (Niiyama '59). It has been shown by cytologists (White '40, '41, '54, Cooper '46, '51, Hughes-Schrader '47, '50) that the XX-Y mechanism was secondarily derived from either an originally XO- or an XY condition by translocation involving an autosome and sex-chromosomes. In the Copepoda, male heterogamety of the XO-type was reported by Heberer ('32), while Beervann ('54) observed female heterogamety of the XO-type. In the Ostracoda, besides the XY-type (*Cyclocypris laevis*, Dietz '58) and XO-type (*Cyclocypris globosa*, Dietz '58), a compound sex-chromosomes was reported in males of 12 species, such as X_{1-6}-Y in *Heterocypris incongruens* (Bauer '40), X_{1-2}-O in *Notodromas monacha* (Dietz '54), X_{1-3}-Y in four species of *Cypris*, *Physocypria kliei*, *Platycypris baueri* and *Scottia browniana* X_{3-6}Y or X_{4-7} Y in *Cyclocypris ovum* and X_{1-3}-Y or $X_{1-3}X_4$-Y in *Cypria exsculpta* and X_{1-3} X_4-Y in *Cypria ophthalmica* (Dietz '58). Similar situation was found to occur also in some species of insects (see Makino's list 1956).

The remarkably cytological feature is the evidence of distance pairing of the XY sex-chromosomes mechanism which was found to exist throughout all the species of the Family Grapsidae so far studied. The distance pairing was also found to occur in the

X_1X_2 and Y sex-chromosomes of *Cervimunida princeps* belonging to the Family Galateidae, as described in the foregoing pages. In none of the cases above noted was the establishment of distance pairing was observed with certainty in the meiotic prophase, since there appear at diakinetic prophase many autosomal chromatic bodies which are indistinguishable in size from X_1, X_2 and Y in the nucleus. In *Anisogammarus annandalei* the association of the X and Y by means of connecting fibres was found in the diakinetic stage. The connecting fibres seem to contract by and by with the advance in stage in the meiotic prophase nucleus and as a result, the two elements come to lie in the equatorial plate assuming an end-to-end conjugation with their long axes at right angle to the plate at metaphase.

Based on the evidence presented above the author considers that the distance pairing of the sex-chromosome at metaphase depends on the degree of contraction of the connecting fibres between the two sex-elements. This is suggested by the fact that the distance between the X and Y pair at metaphase is dissimilar in different species of Family Grapsidae. The distance between the X and Y pair is greatest in *Hemigrapsus sanguineus* (Figs. 105–106) and *Gaetice depressus* (Figs. 127–128), while in *Hemigrapsus penicillatus* (Figs. 112–114) and *Plagusia dentipes* (Figs. 137–140) it ranks next. *Eriocheir japonicus* (Figs. 120–121) and *Pachygrapsus crassipes* (Figs. 97–99) show the smallest distance between them. To the extreme case, the X and Y show no evidence of distance pairing, an example of which was found in *Anisogammarus annandalei* (Fig. 187). Though the X and Y show no evidence of distance pairing in the latter form, the sex-elements are clearly distinguishable from the autosomal elements by the fact that the two differ considerably in size. If the size-difference between the X and Y is very small or nearly identical in magnitude, the X and Y could not be identified among the autosomes in the complex. The case may be found, to the author's view, in the majority of species of the Crustacea in which there is no evidence for the existence of the sex-chromosomes. Generally speaking it is quite impossible to find out in the Crustacea the sex-chromosome by the usual method of alignmental arrangement of homologous chromosomes in a complex, since there are many chromosomes dimishing in size gradually from rod to minute spherule in a complex, and accordingly there is a difficulty in identifying the homologous mates.

Ovalipes punctatus is characterized by the XO-type sex-mechanism. It is remarkable that the X chromosome of the present species always lie in the central position of the equatorial plate, considerably apart from the equatorial plate. The position of the X of this species is nearly identical with that of the X found in several species of the Grapsidae which show the XY-mechanism. The impression is given that the unusual position of the X in *Ovalipes punctatus* is of the ancestral type deviated from the distance pairing of the XY mechanism. It seems very probable to the author that the ancestor of *Ovalipes punctatus* may have had XY-mechanism showing the distance pairng as occurs in many species of the Grapsidae, and that the disappearance of the Y may have occurred in

comparatively later time in the course of evolution, or that Y still remains in the state of a heterochromatin. Based on the above considerations, it is likely that the XY sex-chromosomes occurring in the Grapsidae and the XO sex-chromosome existing in the *Ovalipes punctatus* may have had a similar origin in the evolutionary development.

In contrast to the XO sex-chromosomes as discussed above, the sex-mechanism found in *Tecticeps japonicus* is of a type commonly occurring among insects (see Makino's list. 1956). It seemes apparent that such an XO-mechanism may have resulted from the disappearance of the Y chromosome long ago. The fact that the X chromosome lies far apart from the equatorial plate at metaphase may not be due to the precession of that chromosome in division.

Rather frequently a clear constriction is found to occur in the middle portion of the X chromosome of *Plagusia dentipes* and *Eriocheir japonicus* : this finding seems to suggest a close relation between the XY-mechanism and XX-Y mechanism of sex-chromosomes since such a constriction of the X-chromosome may be due to the existence of a compound nature of the chromosome. If the two components of a compound X are separated there may occur two X's called X_1 and X_2. This is strongly supported by the assumption of White ('40, '41, '54), Cooper ('46, '51), and Hughes-Schrader ('47, '50) who believed that the X_1X_2Y-mechanism was secondarily derived from either an originally XO- or XY-condition by translocation involving an autosome and sex-chromosomes. The X having a constriction as occurred in *Plagusia dentipes* (Figs. 137–140) and *Eriocheir japonicus* (Figs. 120-121), is thick in appearance, while the X-element of XY-type occurring in *Hemigrapsus sanguineus* (Figs. 105–106) is slender in form. The former X chromosome of the former type may be produced as a result of translocation involving an autosome and sex-chromosomes of the latter type.

The evolutionary process of the sex-chromosome, therefore, seems to proceed two ways from the XY-type occurring in *Hemigrapsus sanguineus*. As a result of the disappearance of the Y in a certain mechanism from the XY-mechanism found in *Hemigrapsus sanguineus*, there may be produced the XO-type which occurs in *Ovalipes punctatus*, is one way. And in another way, by the association of a pair of autosomes with the sex-chromosome as occurs in *Hemigrapsus sanguineus*, the XY-mechanism occurring in *Plagusia dentipes* and *Eriocheir japonicus* may result. If the X chromosome of *Plagusia dentipes* is divided into two components, there may be derived an X_1X_2Y-type as found in *Cervimunida princeps*. If the XY chromosomes having a compound nature cast off the Y-element during the evolutionary process, the XO-type mechanism as found in *Tecticeps japonicus* may be produced. The association of the sex-chromosome with a pair of autosomes might occur on the one hand and on the other hand the disappearance of the Y chromosome might occur repeatedly, so the multiple X occurring in the Ostracoda will appear (Bauer '40, Dietz '54, '58).

In conclusion, though the elucidation of the evolutionary process of the sex-

Table 3. The species under study and their chromosome numbers

Decapoda	2*n*	Formula	*n*
Penaeus japonicus	92	14V′+78R′	46
Panulirus japonicus	140	12V′+128R′	70
Cambarus clarkii	192	6V′+186R′	96
Cambaroides japonicus	196	196 rods	98
Nephrops japonicus	164	164 rods	82
Nephropsis carpenteri	152	30V′+122R′	76
Cervimunida princeps	109	18V′+91R′	$53+X_1X_2Y$ (♂I)
Eupagurus ochotensis	254	18V′+236R′	127
Coenobita rugosa	230	230 rods	
Paralithodes camtschatica	208	208 rods	104
Paralithodes platypus	206	18V′+188R′	103
Matuta lunaris	94	4V′+90R′	47
Philyra pisum	114	4V′+110R′	57
Ranina ranina	106	24V′+82R′	53
Macrocheira kaempferi	106	16V′+90R′	53
Ovalipes punctatus	103	4V′+99R′	51+XO (♂I)
Scylla serrata	106	106 rods	53
Telmessus cheiragonus	124	18V′+116R′	62
Atergatis floridus	104	4V′+100R′	52
Pachygrapsus crasssipes	118	118 rods	58+XY (♂I)
Hemigrapsus sanguineus	128	128 rods	63+XY (♂I)
Hemigrapsus penicillatus	138	2V′+136R′	68+XY (♂I)
Eriocheir japonicus	148	148 rods	73+XY (♂I)
Gaetice depressus	152	12V′+140R′	75+XY (♂I)
Sesarma intermedia	102	102 rods	
Plagusia dentipes	106	106 ords	52+XY (♂I)
Isopoda			
Megaligia exotica	72	12V′+60R′	36
Tylos granulata	68	20V′+48R′	34
Cleantiella isops	64	34V′+30R′	32
Idotea japonica	64	30V′+34R′	32
Cymodoce japonica	58	32V′+26R′	29
Tecticeps japonicus	63	27V′+36R′	31+XO (♂I)
Amphipoda			
Anisogammarus annandalei	54	54 rods	26+XY (♂I)

mechanism remains incomplete at present due to the poor data, the impression is given that the sex-chromosomes of the Crustacea exhibit an evolutionary development showing a connection with the taxonomical relationship.

SUMMARY

The present study is divided into three parts. Part I is devoted to the description of the comparative morphology of chromosomes in 33 species of the Decapoda, Isopoda and Amphipoda of Crustacea. The species under study and the number of chromosomes established by the present author are compiled in Table 3.

Part II deals with the chromosome morphology in relation to taxonomical classification of the Crustacea, by reference to the cytological data so far presented by cytologists. The numerical relationship of the chromosomes so far established in the Crustacea is shown

as a summary in Table 1.

The Decapoda, though there occurs a wide range of variation in the number of chromosomes, seems to be differentiable into two classes on the basis of chromosome counts. One class of decapods is characterized by more or less 200 chromosomes, while the other by 100 or thereabout.

In the Isopoda the chromosome number varies rather widely ranging from $2n$, 12 to $2n$, 72. Vandel states that the chromosome number of the true Oniscoidea shows a tendency to increase with the evolution of species, and that two extreme forms show a high chromosome number, while the intermediate species have a smaller number. There are, however, considerable exceptions which show variation from Vandel's rule. In the author's opinion, it is difficult to find any rule for a generalization of the relationship between taxonomical classification and cytological features in the present order.

In the Amphipoda, on the other hand, the counts show comparatively little variation. Though there are some exceptions, it was decided that the fundamental number of chromosome of the Family Gammaridae is 26 (n) and that of the Family Talitridae is 25 (n).

In Part III is discussed the sex-determining mechanism in the Crustacea. It has been reported on the basis of the cytological study that the majority of crustacean species show male heterogamety, while in certain forms female heterogamety occurs. The data so far accumulated are shown in Table 2. Disregarding certain reports open to doubt, one may say that the sex-determining mechanism of the Crustacea is of the XO-, XY- type or modifications thereof. In several species of the Decapoda, Amphipoda and Phyllopoda, the XO- XY and XXY-mechanism was found to occur in the male. In the Copepoda, male heterogamety of an XO-type and female heterogamety of the same type have been reported. In the Ostracoda, a compound sex-chromosome was found to occur in males of 12 species.

The relationship between the X-O, X-Y and XX-Y mechanism was considered on the basis of evolutionary development of the sex-mechanism in the Crustacea in general, and some possible features were discussed of the development of the sex-chromosomes in relation to taxonomy.

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EXPLANATION OF PLATES

PLATE I

Figs. 1– 4. Chromosomes of *Penaeus japonicus*.

1. Polar view of spermatogonial metaphase, 92 (14V′+78R′) chromosomes.

2– 3. Metaphase polar views of primary spermatocytes, 46 bivalents in each.

4. Metaphase polar view of secondary spermatocyt, 46 dyads.

Figs. 5– 9. Chromosomes of *Panulirus japonicus*.

5– 6. Polar views of spermatogonial metaphases, 140 (12V′+128R′) chromosomes in each.

7– 8. Metaphase polar views of primary spermatocytes, 70 bivalents in each. Note three compound ring-tetrads and transvers suturs at middle portions of large bivalents.

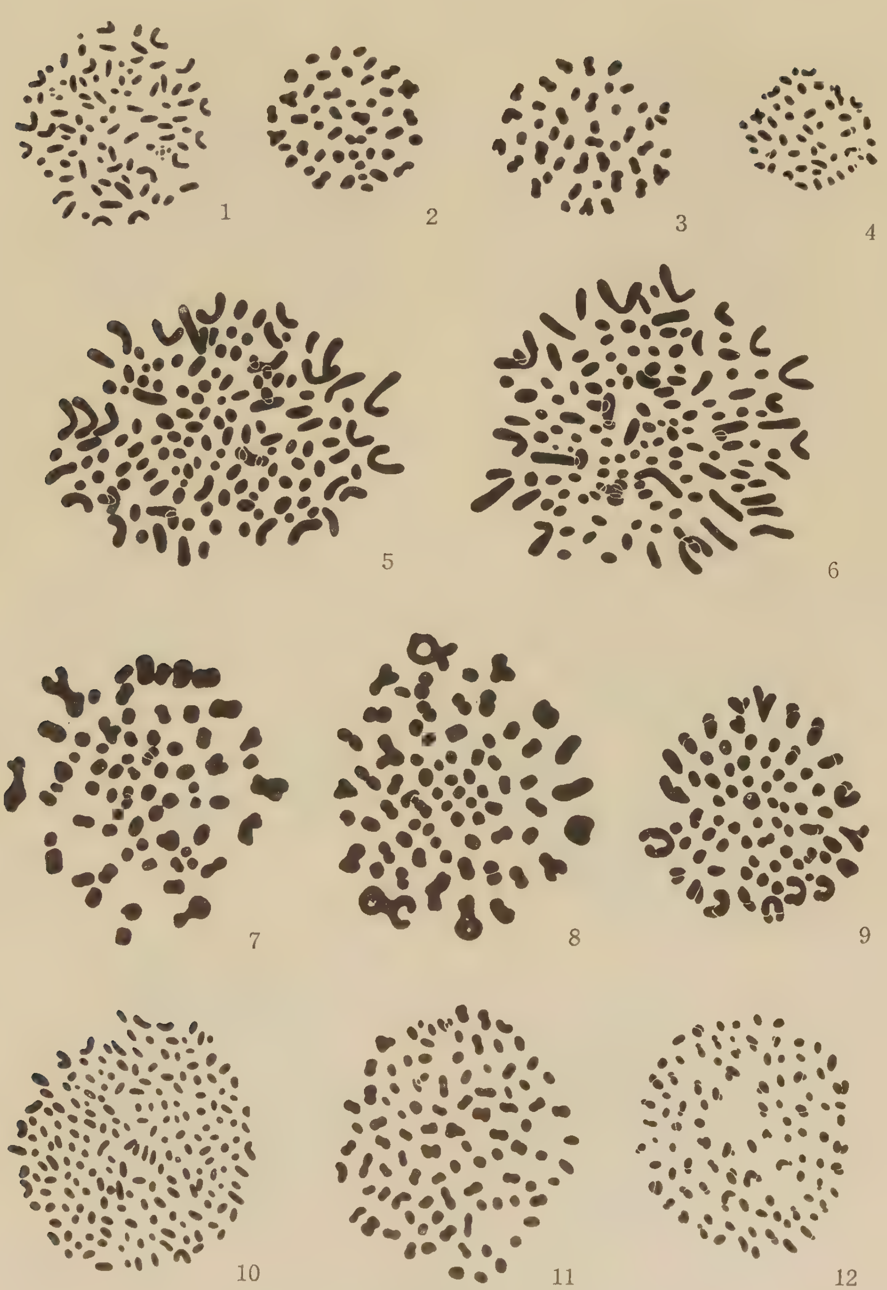
9. Metaphase polar view of secondary spermatocyte, 70 dyads.

Figs. 10–12. Chromosomes of *Cambarus clarkii*.

10. Polar view of spermatogonial metaphase, 192 (6V′+186R′) chromosomes.

11. Metaphase polar view of primary spermatocyte, 96 bivalents.

12. Metaphase polar view of secondary spermatocyte, 96 dyads.



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PLATE II

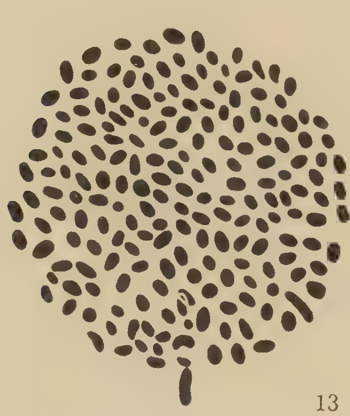
Figs. 13–18. Chromosomes of *Cambaroides japonicus*.

13–14. Spermatogonial complexes, 196 rod-shaped chromosomes in each.

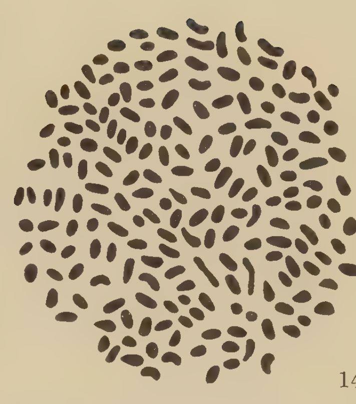
15–16. Metaphase polar views of primary spermatocytes, 98 bivalents in each. Note transverse sutures at middle portions of bivalents.

17. Metaphase polar view of secondary spermatocytes, 98 dyads.

18. One of two daughter chromosome garnitures at anaphase of the secondary divisions, containing 98 monads.



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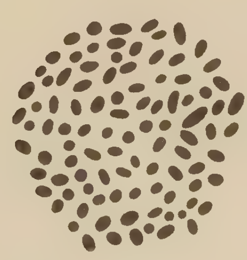
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PLATE III

Figs. 19–21. Chromosomes of *Nephrops japonicus*.

19. Polar view of spermatogonial metaphase, 164 (all rod) chromosomes.

20. Metaphase polar view of primary spermatocyte, 82 bivalents. Note quadripartite structure of bivalents.

21. Side-view of primary division at early anaphase.

Figs. 22–25. Chromosomes of *Nephropsis carpenteri*.

22. Polar view of spermatogonial metaphase, 152 (30V′+122R′) chromosomes.

23–24. Metaphase polar views of primary spermatocytes, 76 bivalents in each. Note quadripartite and hexapartite structure of bivalents.

25. Side-view of primary division at early anaphase, Note quadripartite, hexapartite and octopartite structure of bivalents.

PLATE III



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PLATE IV

Figs. 26–36. Chromosomes of *Cervimunida princeps*.

26. Metaphase plate of spermatogonium, 109 (18V′+91R′) chromosomes.

27–28. Polar views of metaphase chromosomes of primary spermatocytes, 53 bivalents and X_1X_2Y chromosomes in each.

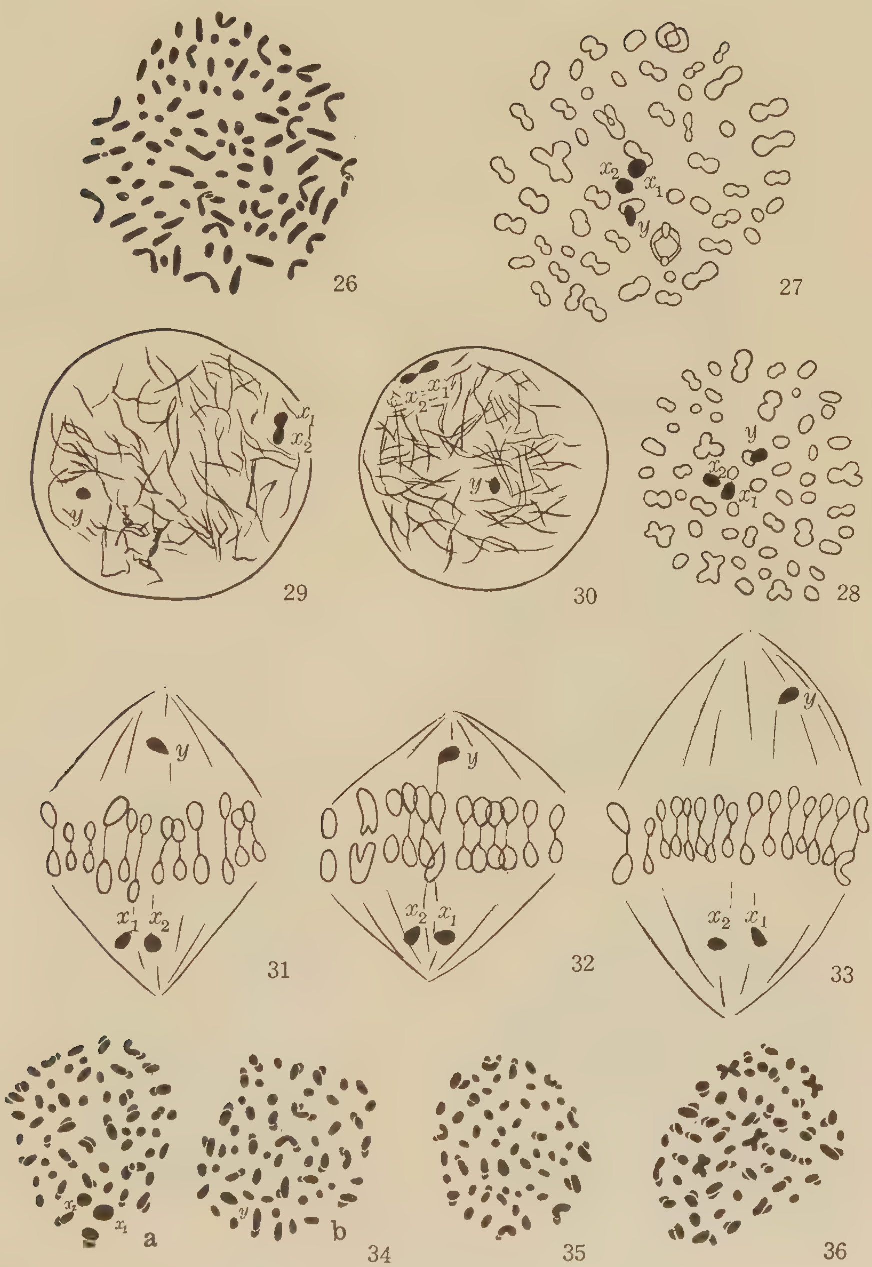
29–30. Nuclei in leptotene stage, showing heteropycnotic bodies consisting of X_1X_2 and Y in deep black.

31–33. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X_1X_2-Y in each.

34 a–b. Two daughter complexes of the equatorial plate of a primary spermatocyte at anaphase.

35. Metaphase of a secondary spermatocyte, containing 54 chromosomes.

36. The same, with 55 chromosomes.



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PLATE V

Figs. 37–40. Chromosomes of *Eupagurus ochotensis.*

37. Polar view of spermatogonial metaphase, 254 (18V′+236R′) chromosomes.

38–39. Metaphase polar views of primary spermatocytes, 127 bivalents in each.

40. Metaphase polar view of secondary spermatocyte, 127 dyads.

Fig. 41. Spermatogonial chromosomes at metaphase of *Coenobita rugosa,* 230 rod-shaped chromosomes.

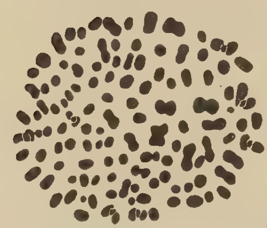
Figs. 42–43. Spermatogonial garnitures of *Paralithodes camtschatica* at metaphase, 208 chromosomes of rod shaped in each.



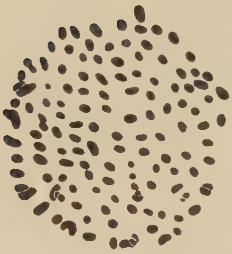
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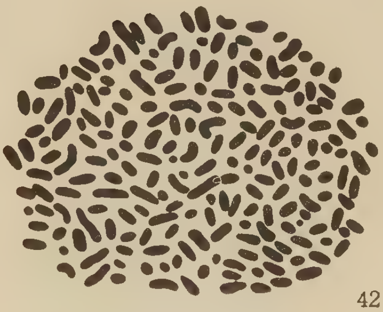
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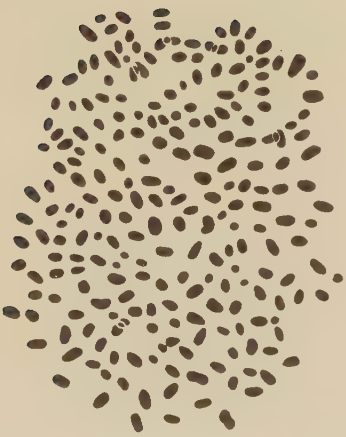
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PLATE VI

Figs. 44–47. Chromosomes of *Paralithodes camtschatica.*

44–45. Metaphase polar views of primary spermatocytes, 104 bivalents having transverse sutures at their middle portions.

46–47. Metaphase polar views of secondary spermatocytes, 104 dyads in each.

Figs. 48–51. Chromosomes of *Paralithodes platypus.*

48. Polar view of spermatogonial metaphase, 206 (18V′+188R′) chromosomes.

49–50. Metaphase polar views of primary spermatocytes, 103 bivalents in each.

51. Metaphase polar view of secondary spermatocyte, 103 dyads.



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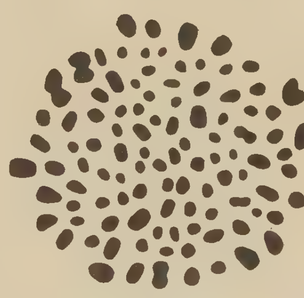
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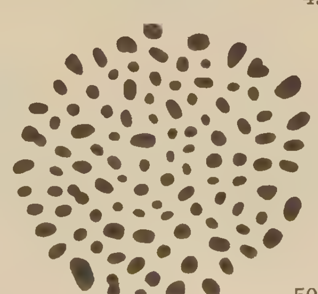
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PLATE VII

Figs. 52–56. Chromosomes of *Matuta lunaris*.

52–53. Polar views of spermatogonial metaphases, 94 (4V′+90R′) chromosomes.

54–55. Metaphase polar views of primary spermatocytes, 47 bivalents in each.

56. Metaphase polar view of secondary spermatocyte, 47 dyads.

Figs. 57–60. Chromosomes of *Philyra pisum*.

57. Polar view of spermatogonial metaphase, 114 (4V′+110R′) chromosomes.

58–59. Metaphase polar views of primary spermatocytes, 57 bivalents in each.

60. Metaphase polar view of secondary spermatocyte, 57 dyads.

Figs. 61–63. Chromosomes of *Ranina ranina*.

61. Polar view of spermatogonial metaphase, 106 (24V′+82R′) chromosomes.

62. Metaphase polar view of primary spermatocyte, 53 bivalents.

63. Metaphase polar view of secondary spermatocyte.

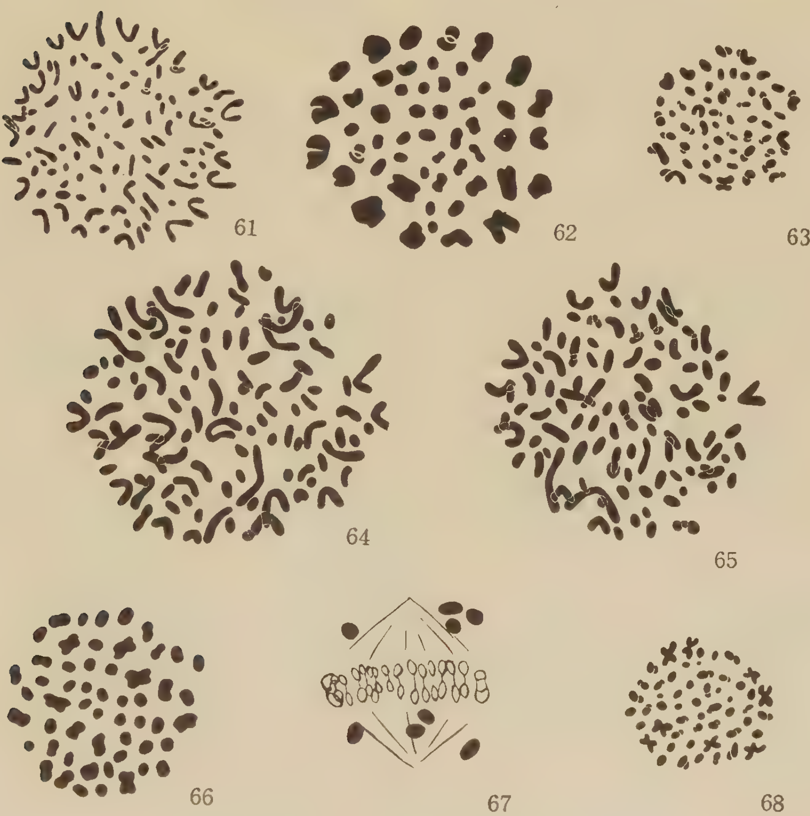
Figs. 64–68. Chromosomes of *Macrocheira kaempferi*.

64–65. Polar views of spermatogonial metaphases, 106 (16V′+90R′) chromosomes.

66. Metaphase polar view of primary spermatocyte, 53 bivalents.

67. Metaphase side view of primary spermatocyte, indicating chromatoid bodies in deep black.

68. Metaphase polar view of secondary spermatocyte, 53 dyads.



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PLATE VIII

Figs. 69–78. Chromosomes of *Ovalipes punctatus*.

69–70. Polar views of spermatogonial metaphases, 103 (4V′+99R′) chromosomes in each.

71–72. Metaphase polar views of primary spermatocytes, 51 bivalents and an X chromosome in each.

73–74. Side views of metaphase chromosomes of primary spermatocytes, indicating an X chromosome in precession in each.

75–76. Metaphase polar views of secondary spermatocyte, containing 51 chromosomes in each.

77–78. The same, with 52 chromosomes.

Figs. 79–83. Chromosomes of *Scylla serrata*.

79–80. Polar views of spermatogonial metaphases, 106 rod-shaped chromosomes in each. 79 : early spermatogonium. 80 : late spermatogonium.

81–82. Metaphase polar views of primary spermatocytes, 53 bivalents in each.

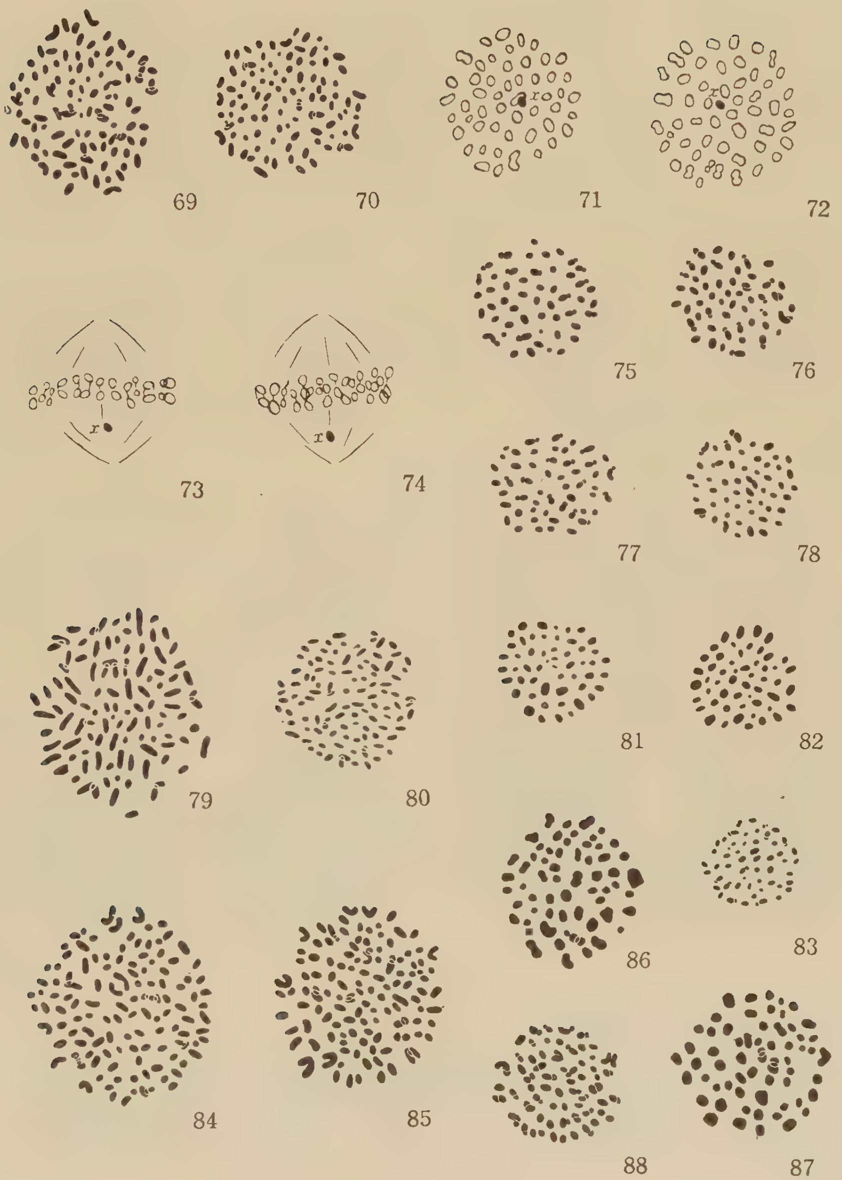
83. Metaphase polar views of secondary spermatocyte, 53 dyads.

Figs. 84–88. Chromosomes of *Telmessus cheiragonous*.

84–85. Polar views of spermatogonial metaphases, 124 (8V′+116R′) chromosomes in each.

86–87. Metaphase polar views of primary spermatocytes, 62 bivalents in each.

88. Metaphase polar view of secondary spermatocyte, 62 dyads.



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PLATE IX

Figs. 89–92. Chromosomes of *Atergatis floridus*.

89. Polar view of spermatogonial metaphase, 104 (4V′+100R′) chromosomes.

90–91. Metaphase polar views of primary spermatocytes, 52 bivalents in each.

92. Metaphase polar view of secondary spermatocyte, 52 dyads.

Figs. 93–100. Chromosomes of *Pachygrapsus crassipes*.

93. Polar veiw of spermatogonial metaphase, 118 (all rod) chromosomes.

94–96. Metaphase polar views of primary spermatocytes, 58 bivalents and XY chromosomes in each.

97–99. Side views of metaphase chromosomes of primary spermatocyte, indicating distance pairing of X-Y in each.

100. Metaphase polar view of a secondary spermatocyte, 59 dyads.

Figs. 101–108. Chromosomes of *Hemigrapsus sanguineus*.

101–102. Polar views of spermatogonial metaphases, 128 (all rod) chromosomes in each.

103–104. Metaphase polar views of primary spermatocytes, 63 bivalents and XY chromosomes in each.

105–106. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.

107–108. Metaphase polar views of secondary spermatocytes, 64 dyads in each.


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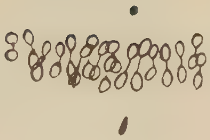

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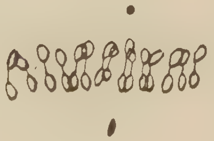

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PLATE X

Figs. 109–115. Chromosomes of *Hemigrapsus penicillatus*.

109. Polar view of spermatogonial metaphase, 138 (2V′+136R′) chromosomes.

110–111. Metaphase polar views of primary spermatocytes, 68 bivalents and XY chromosomes in each.

112–114. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.

115. Metaphase polar view of secondary spermatocyte, 69 dyads.

Figs. 116–122. Chromosomes of *Eriocheir japonicus*.

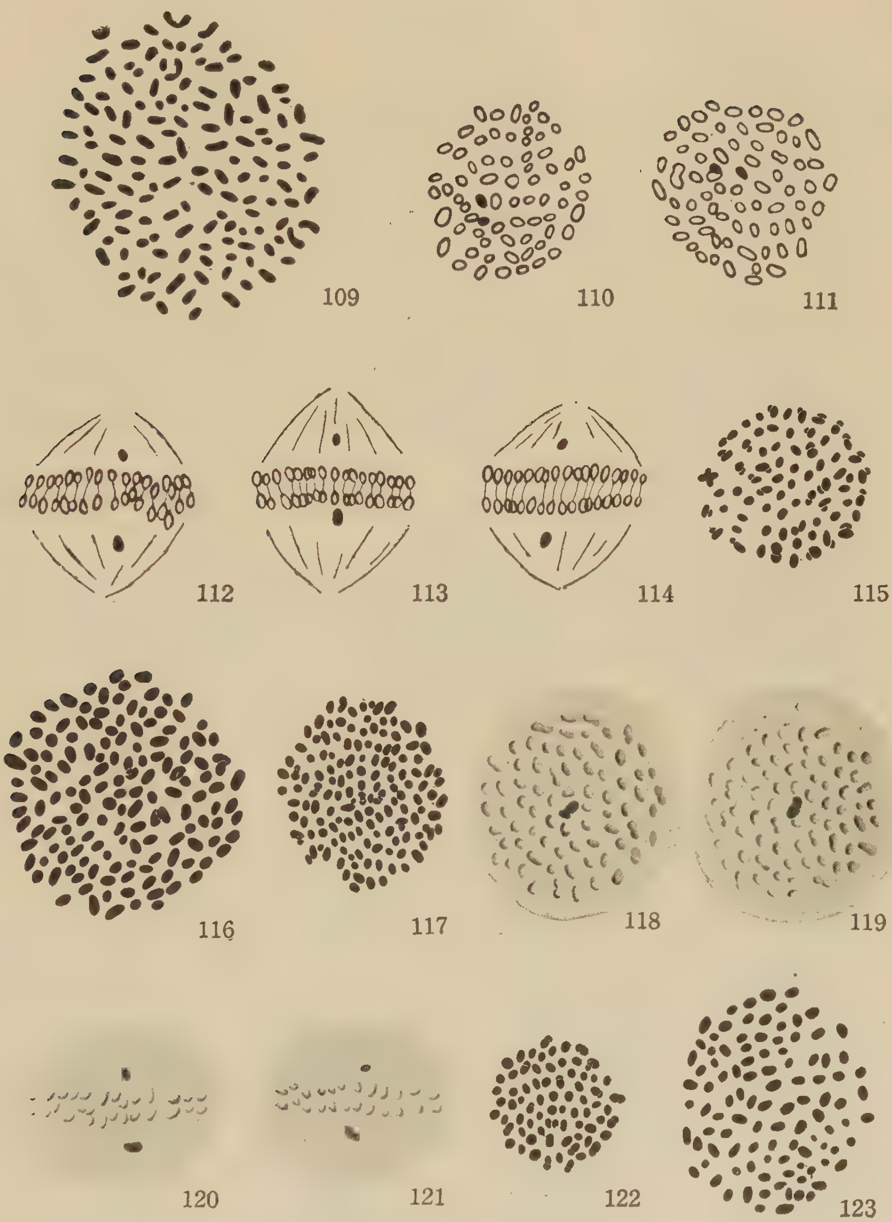
116–117. Polar views of spermatogonial metaphases, 148 (all rod) chromosomes in each 116 : early spermatogonium. 117 : late spermatogonium.

118–119. Metaphase polar views of primary spermatocytes, 73 bivalents and XY chromosomes in each.

120–121. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.

122. Metaphase polar views of secondary spermatocyte, 74 dyads.

Fig. 123. Metaphase polar view of spermatogonial chromosomes of *Sesarma intermedia*, containing 102 rod-shaped chromosomes.



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PLATE XI

Figs. 124–129. Chromosomes of *Gaetice depressus.*

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125–126. Metaphase polar views of primary spermatocytes, 75 bivalents and XY complex in each.

127–128. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing X-Y in each.

129. Metaphase polar view of secondary spermatocyte, 76 dyads.

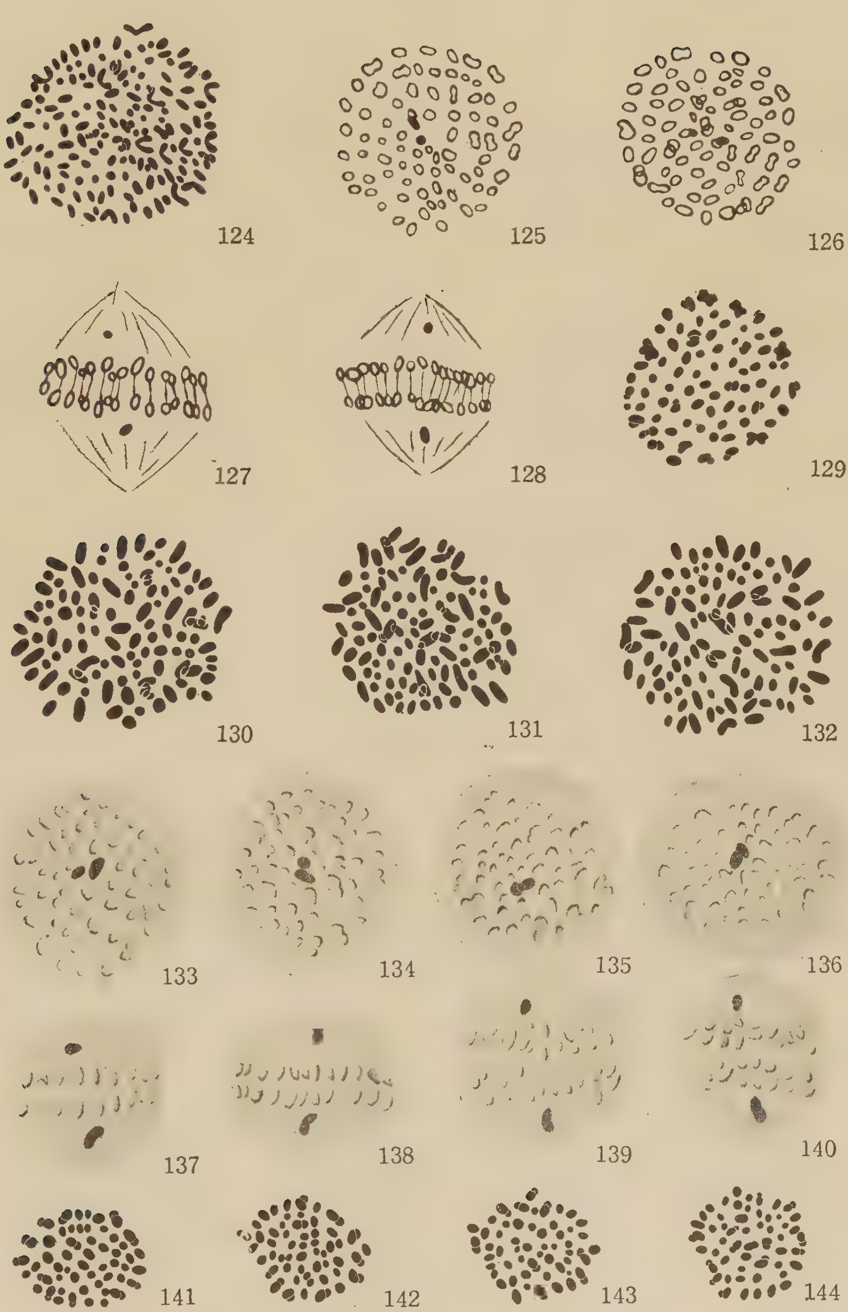
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133–136. Metaphase polar views of primary sperm .tocytes, 52 bivalents and XY complex in each.

137–140. Side views of metaphase chromosomes of primary spermatocytes, indicating distance pairing of X-Y in each.

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PLATE XII

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146–147. Metaphase polar views of primary spermatocytes, 36 bivalents in each.

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Figs. 149–152. Chromosomes of *Tylos granulata*.

149. Polar view of spermatogonial metaphase, 68 (20V′+48R′) chromosomes.

150–151. Metaphase polar views of primary spermatocytes, 34 bivalents in each.

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Figs. 153–155. Chromosomes of *Cleantiella isops*.

153. Polar view of spermatogonial metaphase, 64 (34V′+30R′) chromosomes.

154. Metaphase polar view of primary spermatocyte, 32 bivalents.

155. Metaphase polar view of secondary spermatocyte, 32 dyads.

Figs. 156–158. Chromosomes of *Idotea japonica*.

156. Polar view of spermatogonial metaphase, 64 (30V′+34R′) chromosomes.

157. Metaphase polar view of primary spermatocyte, 32 bivalents.

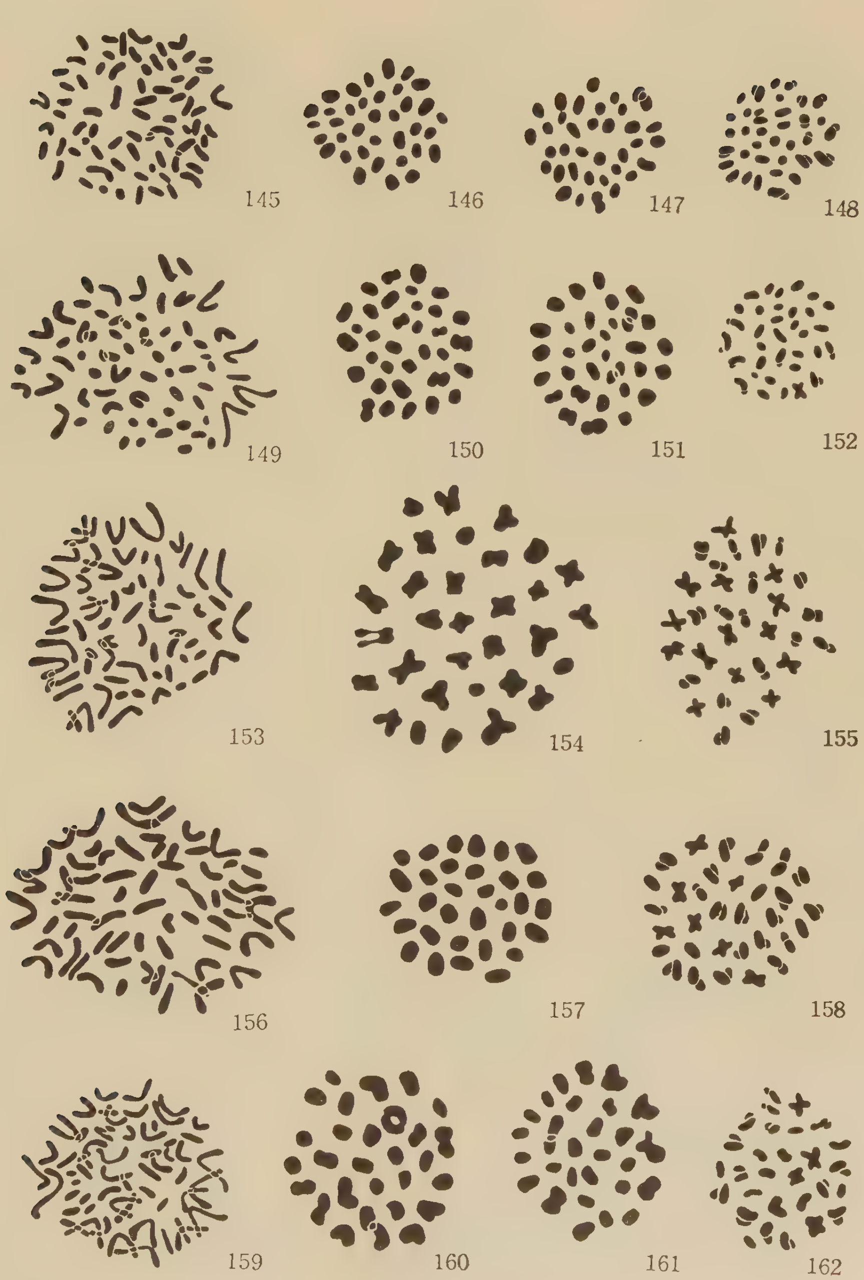
158. Metaphase polar view of secondary spermatocyte, 32 dyads.

Figs. 159–162. Chromosomes of *Cymodoce japonicus*.

159. Polar view of spermatogonial metaphase, 58 (32V′+26R′) chromosomes.

160–161. Metaphase polar views of primary spermatocytes, 29 bivalents in each.

162. Metaphase polar view of secondary spermatocyte, 29 dyads.



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PLATE XIII

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163–164. Metaphase chromosomes of spermatogonia, 63 (27V′+36R′) autosomes and an X chromosome in each.

165. Serial alignment of the paired chromosomes from Fig. 163.

166. Nucleus in diakinesis stage, indicating an X chromosome in deep black.

167–168. Polar views of metaphase chromosomes of primary spermatocytes, 31 bivalents and an X-chromosome in each.

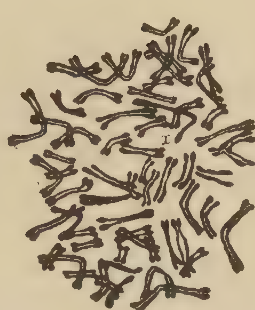
169. Metaphase chromosomes of secondary spermatocyte containing an X-chromosome.

170–171. Side views of metaphase chromosomes of primary spermatocytes.

172. Side view of early anaphase chromosomes of primary spermatocyte.



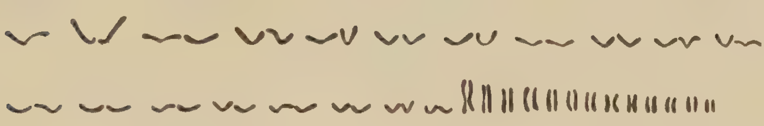
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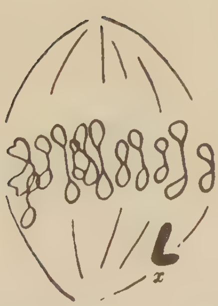
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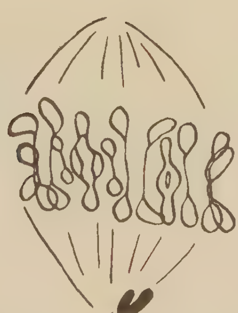
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PLATE XIV

Figs. 173–191. Chromosomes of *Anisogammarus annandalei.*

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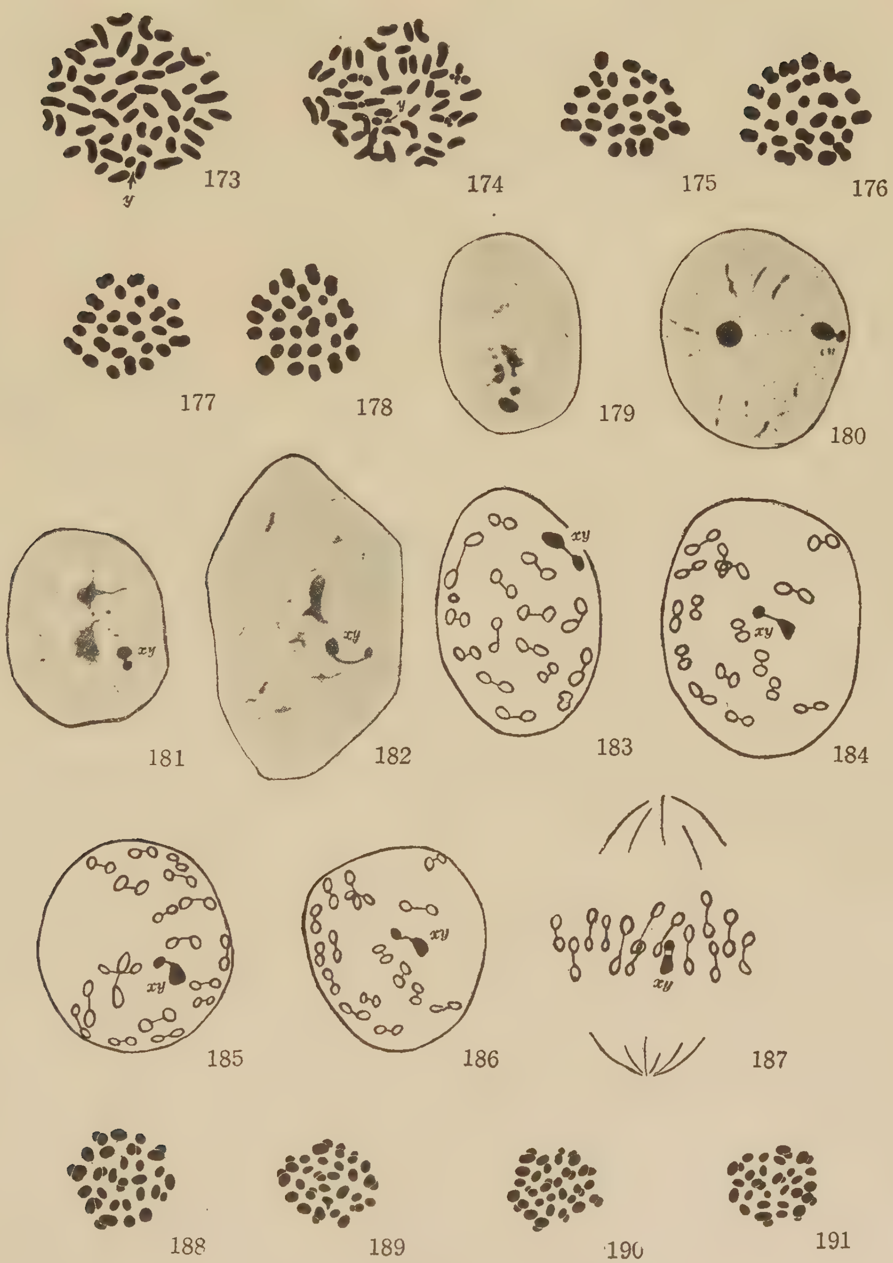
175–178. Polar views of metaphase chromosomes of primary spermatocyte, 27 bivalents in each.

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188–191. Pol.r view of metaphese chromosomes of secondary spermatocytes, 27 dyads in each.



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CYTOLOGICAL STUDY ON SOME JAPANESE SPECIES OF RHODOMELACEAE

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CONTENTS

INTRODUCTION

The first cytological study in the species belonging to the Family Rhodomelaceae is that which was made by Yamanouchi (1906) with *Polysiphonia violacea.* After that there have been published four papers dealing with the same subject by three other investigators, *viz.*, Kylin (1914, 1923), Westbrook (1928), and Austin (1956).

The following table shows the chromosome numbers of nine Rhodomelaceous species counted by these workers.

Table I

Species	Chromosome number		Author
	n	2n	
Polysiphonia violacea	20	40	Yamanouchi, 1906
P. nigrescence	20		Kylin, 1923
〃 〃	30	60	Austin, 1956
P. elongata	37		Austin, 1956
P. lanuosa	27		Austin, 1956
Chondria dasyphylla	18-19	40	Westbrook, 1928
Laurencia hybrida	31		Austin, 1956
L. pinnatifida	29	40	Kylin, 1923
〃 〃			Austin, 1956
Rhodomela virgata	20		Kylin, 1914
Rh. confervoides		40	Kylin, 1914
〃 〃	32		Austin, 1956

Since none of the Japanese species of Rhodomelaceae has ever been studied cytologically, the writers undertook the present study in 1957 with several species, and fortunately could obtain satisfactory results in the following four species: *Polysiphonia japonica* HARV., *Chondria crassicaulis* HARV., *Laurencia papillosa* HARV. and *L. obtusa* LAMOUR. var. *majuscula* HARV. So the writers wish to report here the results of their observations. On this opportunity the writers acknowledge their indebtedness to Professor J. Tokida for his kindness in reading the manuscript, and in giving valuable advices.

MATERIAL AND METHOD

Of the four species studied, *Polysiphonia japonica* and *Chondria crassicaulis* were collected several times in 1957 at Nanaehama near Hakodate, the former in the period ranging from late September to the middle of December and the latter from early September to the middle of December. The collected materials were brought to the laboratory and kept alive for some time in glass vessels containing sea water. On the other hand, the remaining two species belonging to the genus *Laurencia* were collected by the junior writer on the 2nd and the 3rd of August 1957 at Okinoshima, Sukumo City in Kôchi prefecture, and fixed on the spot. The fixed materials kept in 75% alcohol were brought to the

laboratory.

Fixation of the materials was done with the following seven fixatives applied for various lengths of time respectively (Table II). Sections were cut 4–7μ in thickness by paraffin method and stained with Heidenhein's haematoxylin.

Table II

Material	Fixing fluid	Fixing hours
Polysiphonia japonica	Yamanouchi's fluid	3
	Abe's fluid	3
	Modified Yamanouchi's fluid	5
	Modified Abe's fluid	5
Chondria crassicaulis	Abe's fluid	3
	Tahara's fluid	3–4
	Modified Abe's fluid	3
	Modified Yamanouchi's fluid	3
Laurencia papillosa	Fluid consisting of chromic, picric, and acetic acid and formalin	10
Laurencia obtusa	Fluid consisting of chromic and acetic acid and formalin	1
	Modified Allen's fluid	10

The formulas of the fixing fluids employed are as follows :

1) Yamanouchi's fluid (Yamanouchi, 1909)

2% osmic acid	1.0 cc
1% chromic acid	5.0 cc
Glacial acetic acid	2.0 cc
Sea-water	11.0 cc

(Yamanouchi used 1% solution of osmic acid in various volumes)

2) Modified Yamanouchi's fluid

2% osmic acid	1.0 cc
1% chromic acid	5.0 cc
Glacial acetic acid	1.0 cc
Sea-water	11.0 cc

3) Abe's fluid (Abe, 1933)

A) Stock solution of chromic acid (sea-water 98 cc, saturated water solution of chromic acid 2 cc)	50.0 cc
Sea-water	50.0 cc
2% osmic acid	5.0 cc
Glacial acetic acid	2.5 cc
B) Saturated solution of picric acid	50.0 cc

	Glacial acetic acid	5.0 cc
	Chromic acid	1.0 cc
C)	Saturated solution of picric acid	25.0 cc
	40% formalin	25.0 cc
	Urea	0.5 cc

These three solutions were mixed just before use at the ratio A : B : C = 2 : 1 : 1.

4) Modified Abe's fluid

Each of the solutions (A) and (B) of Abe's fluid is adjusted to contain 1.0 cc of glacial acetic acid in this modification.

5) Tahara's fluid (Tahara, 1929)

2% osmic acid	5.0 cc
2% chromic acid	70.0 cc
Glacial acetic acid	2.5 cc
Sea-water	30.0 cc

6) Fluid consisting of chromic, picric and acetic acid and formalin

Stock solution of chromic acid (2 cc of saturated water solution of chromic acid in 98 cc of sea-water)	5.0 cc
Saturated picric acid	50.0 cc
Formalin	5.0 cc
Glacial acetic acid	2.0 cc

7) Fluid consisting of chromic and acetic acid and formalin

Stock solution of chromic acid (2 cc of saturated water solution of chromic acid in 98 cc of sea-water)	5.0 cc
Glacial acetic acid	5.0 cc
Formalin	10.0 cc
Sea-water	50.0 cc

DESCRIPTION OF THE RESULTS OBSERVED

Polysiphonia japonica HARV.

In this species, the nuclear divisions in the somatic and reproductive cells of the tetrasporophyte and the gametophytes, female and male, were observed. The materials were fixed at various times between 7 p.m. and 4 a.m., and the nuclei were found dividing in those fixed between 9 p.m. and 3 a.m. Three hours in the middle of the night between 11 p.m. and 2 a.m. were best for fixing to obtain a good number of dividing nuclei. The fluids recommended by Abe (1933) and Yamanouchi (1909), *viz.*, Nos. 1 and 3 of the above listed formulas, gave satisfactory results in this species, but the writers' modifications, *viz.*,

Nos. 2 and 4, which contain a smaller amount of glacial acetic acid than the former were much better in bringing forth good nuclear figures in both somatic and reproductive cells.

a) Somatic division. All stages of nuclear divisions in somatic cells of both tetrasporic and sexual plants were observed at the terminal portions of the thallus. Colourless hair cells on upper branches are always uninucleate and good for counting the chromosome number in metaphase. Somatic cells in other parts of the thallus are usually uninucleate but sometimes multinucleate. The hair cells and the multinucleate cells were generally not good for the present purposes on account of small size of their nuclei. So the following descriptions are based on larger nuclei in the cells at or near the thallus apices.

Nucleus in early prophase shows a network structure; soon it begins to produce small chromatin granules on the net. These granules become larger in size, while chromatin threads become more slender and gradually disappear. Nucleus in later prophase contains single nucleolus together with well-stained chromatin granules which were called prochromosomes by Yamanouchi (1906). In early metaphase the nuclear membrane and the nucleolus disappear. The number of chromosomes was counted at this state. It was twenty in both female and male plants and forty in tetrasporic plants. In *Polysiphonia violacea*, Yamanouchi (1906, p. 408) observed at the metaphase of somatic division deeply stained centrosome-like bodies which were described as "conspicuous at the poles just before the spindle is formed." Such bodies could not be found in *P. japonica* by the present writers in spite of careful observations. The spindle fibers were not clearly seen in most cases, but sometimes they were visible in a faintly stained material. In anaphase, long or short spindle fibers attached to or detached from chromosomes were found between two groups of daughter chromosomes. After the chromosomes disappeared and the daughter nuclei were produced in telophase, a somewhat vacuolated structure appeared in the equatorial plate of the cell and the cell membrane was formed there perpendicularly to the direction of nuclear division. In Pl. I, Figs. 3–9 are shown the nuclear divisions in the hair cells of sexual and asexual plants.

b) Nuclear division in tetrasporangia. Tetrasporangia make their appearance first near the top of branches and then gradually in lower portions of the thallus. They are cut off from the pericentral cells as follows. A pericentral cell is divided vertically into two cells, of which the inner one is then divided transversely into two *i.e.*, into tetrasporangium mother cell and its stalk cell. The sporangium mother cell soon increases in size and becomes oval in shape. Its nucleus in early prohase has usually one, but rarely two, nucleoli. The nucleus sometimes looks vacuolated or reticulated in structure because of unequal staining of its content. Then, slender chromatin threads appear at one side of the nuclear cavity, and next the nuclear cavity becomes filled with chromatin threads. Existence of chromatin threads in early prohase was clearly recognized in the materials fixed with Abe's fluid. In diakinesis stage of a nucleus, in which the nucleolus had already disappeared, the writers could count 20 gemini which were V-, O-, or rod-shaped. These chromosomes gradually became spherical in shape and somewhat more deeply stained. In metaphase, the nuclear

membrane disappeared completely, and the nuclear cavity which had usually been stained light blue in prophase became colourless and indistinguishable from the surrounding cytoplasm of the sporangium, so that the boundary of the nucleus was quite indistinct. Well-stained small granules, which made their appearance in the cytoplasm of a sporangium when the latter's nucleus was in the prophase of the first division, do not disappear even in metaphase. Hence the chromosomes in metaphase were sometimes apt to be confounded with these granules. Spindle fibers were visible in the materials fixed with Abe's fluid, but centrosome-like bodies at the spindle poles could not be observed in any material. As soon as the chromosomes are drawn to each pole, the second nuclear division sets in as shown in Pl. II, Figs. 11–13. The chromosomes which appeared in the second division are much smaller as compared with those in the first division, but in some cases 20 chromosomes are clearly counted in metaphase. The axes of the second nuclear division are perpendicular to each other. In anaphase of the second division, the outline of the original nuclear membrane comes into view again. The four groups of grand-daughter chromosomes reach the periphery of the original nuclear cavity and remain there for a while as small granules. Then the chromosomes lose their individual outlines and the chromatin network makes its appearance. At this stage a large nucleolus-like body suddenly appears in the center of the nucleus. The nucelous-like body is occasionally broken into small pieces. The cytoplasmic portion of the tetrasporangium shows an alveolar structure in late anaphase of the second division. Prior to this, cleavage furrows are fomred at the periphery of the sporangium. The furrows sometimes make their appearance as early as in the metaphase of the first nuclear division. They gradually grow inward and eventually divide the cytoplasm of the sporangium into four equal parts or young tetraspores, each of which contain one of the four daughter nuclei produced as a result of the nuclear division described above. The alveolar structure of the young tetraspores disappears, and numerous well-stained granular chromatophores are produced at the periphery of the spore. The nucleus in the center of each tetraspore becomes much smaller in size and its chromatin networks fade away. In the meanwhile, the chromatophores change their form and become rod-like or elliptical in shape and are somewhat scattered in the inner cytoplasmic portion of the mature spores.

c) **Nuclear division in spermatogenesis.** Antheridia are formed on short branches at or near the tips of the main filaments. The antheridium is composed while young of a few monosiphonous cells, but later it becomes polysiphonous, oval in outline, being composed of a central siphon surrounded by numerous small cells which have been formed in the following manner. The central siphon gives rise laterally to two-celled short branches, the basal cell of which is larger than the apical. The latter cell is the spermatangium mother cell and it cuts off two spermatangia by oblique walls. The antheridia were easily fixed well for the observation of nuclear divisions with all the above described fixing solutions. The nuclei were much smaller than those in the somatic divisions, but it was possible to count clearly 20 chromosomes in metaphase. Each group of the chromosomes which was drawn to one pole in telophase of the spermatangium mother cell becomes the nucleus

of the spermatangium without losing the outlines of the chromosomes, and thus one spermatium containing 20 chromosomes is produced in each spermatangium.

d) Nuclear division in the development of procarp and gonimoblast. The procarp initial cell is born on the subapical segment of the short apical hairy portion of a branch. The hair is converted from one of several cells cut off from the apical cell or it arises directly from the undivided apical cell itself. The subapical segment of the hair produces five pericentral cells, of which the innermost one becomes the initial of the procarp. As shown in Plate IX, Figs. A and C, the procarp initial cell can be recognized from early by its larger size as compared with nearby vegetative cells. It cuts off a mother cell of the carpogonial branch, and finally a four-celled carpogonial branch is produced. The supporting cell of this branch, which is no other than the initial cell of the procarp, cuts off two neutral cells, one near its base and the other at its side. This latter cell divides into two cells, each of which usually remains simple but sometimes abnormally develops into a row of several neutral cells. Pl. III, Fig. 6 shows a procarp just before fertilization. The trichogyne was always found to be destitute of a nucleus, though Yamanouchi (1906, p. 413) mentioned the existence of the trichogyne nucleus in *Polysiphonia violacea*. Pl. III, Fig. 3 shows the nuclear division in metaphase in an initial cell of the procarp. The number of chromosomes counted in the cells was exactly or approximately 20. The nucleus of a spermatium which has entered into the trichogyne is deeply stained with haematoxylin and is occasionally seen as a mass of small chromatin granules. However, during the period from the attachment of a spermatium to a trichogyne till the appearance of a fertilized nucleus in prophase in the carpogonium, the cells of a carpogonial branch are usually stained so dark that it is difficult to observe their nuclei. Therefore the process of fusion between the female and male gamete nuclei in a carpogonium could not be observed. On the other hand, the diploid nuclei in later prophase and early metaphase were frequently observed in the carpogonium (Pl. X, Figs. C–F). The diploid nucleus in prophase was already found to have chromatin granules on the reticulum ; those granules in later prophase were about 40 in number. Then the nucleus increased its size and passed into early metaphase. At this time the nuclear membrane was invisible and the chromosomes, so far as the writers have observed, attained their maximum size as they did also in the division of somatic cells, tetrasporangia, and of spermatangia. In the first mitosis of the diploid nuclei, Yamanouchi (1906, p. 417) observed in his plant two kinds of chromosomes differing from each other in size ; he believed that some of the smaller ones might have come certainly from the male nucleus. However, the writers could not recognize in their material any difference in size among the chromosomes in metaphase of the diploid nucleus. The chromosomes in early metaphase of the fertilized nucleus were 40 in number. After fertilization, the supporting cell of the carpogonial branch increases rapidly in size and soon becomes pear-shaped containing two nuclei (Pl. X, Fig. A). When the diploid nucleus in the carpogonium enters into prophase, the supporting cell divides into two, the upper one of which becomes an auxiliary cell (Pl. X, Fig. D). As soon as the diploid nucleus

enters into early metaphase, the connection between the auxiliary cell and the carpogonium is accomplished (Pl. X, Fig. F). The nucleus of the carpogonium in resting stage is small and well stained ; the auxiliary cell contains one large faintly stained nucleus in the center, which is no doubt one of the daughter nuclei of the fertilized carpogonium nucleus, and one small rod-shaped nucleus, which must be haploid, in the periphery. The auxiliary cell and the supporting cell soon fuse with each other, and the connecting pit between the supporting cell and the central siphon becomes somewhat broadened. The nucleus of the supporting cell occupies the basal portion of the fusion cell. The nucleolus of the resting diploid nucleus is attached to a large globular body which contains minute granules (Pl. IV, Figs. 2 & 3). These granules soon form a reticulum (Pl. IV, Fig. 4). Then the globular body disappears and the nuclear cavity becomes filled with the reticulum (Pl. IV, Fig. 5). The neighboring neutral cells fuse with each other and with the large fusion cell which was formed by the coalescence of the auxiliary cell and the supporting cell. Several haploid nuclei derived from the fusion of the neighboring cells occupy the basal portion of the central cell. The diploid nucleus soon begins its first division, and 40 chromosomes are easily counted in its metaphase. Spindles and centrosome-like bodies were not observed. The daughter nuclei derived from the diploid nucleus undergo successive divisions simultaneously. The gonimoblast-initials arise from the central cell as stated by Yamanouchi (1906) in *Polysiphonia violacea* but not directly from the auxiliary cell as stated by Kylin (1923) in *P. nigrescens*. As Yamanouchi says, the nucleus in each lobe of a gonimoblast usually divides once more and a carpospore is cut off terminally from each lobe, but sometimes the nucleus divides several times in each lobe as shown in Pl. IV, Figs. 11–14 before the carpospores are produced. The nucleus in the spore usually contains one or two nucleoli but rarely several nucleolus-like bodies (Pl. IV, Figs. 13–15). After the spores have been discharged, the walls of the emptied sporangia are seen remaining in the cystocarpic cavity (Pl. IV, Fig. 15).

Chondria crassicaulis HARV.

The tetrasporangial specimens of the present species were fixed in the middle of the night to obtain good figures of dividing nuclei. For the somatic nuclear division, the modified Abe's fluid gave the best result; for the maturation division of the tetrasporangia, Tahara's, Yamanouchi's and Abe's fluids gave good results. On the other hand, Allen's modification of Bouin's fluid and the fluid composed of formalin, glacial acetic acid and alcohol in a formula recommended by Westbrook (1928) were also tried for fixing the present species, but they proved unsuitable for the writer's purpose because the nuclei fixed with these fluids were stained too dark.

a) Somatic division. Somatic nuclear divisions can be observed in the cortical cells of the terminal portion of the branches and branchlets, especially in those at the growing apex. A resting nucleus usually has a single central nucleolus, but rarely two nucleoli, and a few chromatin granules. Westbrook (1928) stated that in *Chondria dasy-*

phylla the fragmentation of nucleoli was commonly observed in resting somatic nuclei and it was very marked in the axial cell. However, such a phenomenon could not be observed in the writers' material. The nucleolus disappears in later prophase. In metaphase, the chromosomes can be counted as 40 in number. In the material fixed with Abe's fluid, side views of the dividing nuclei in metaphase and anaphase could be observed freqeuntly. The spindle fibers were distinctly visible in anaphase (Pl. V, Figs. 5 & 6), but centrosome-like bodies were not observed. After the nuclear division was completed, the cell division generally followed, but, rarely, it did not. Multinucleate cells containing 2 to 5 nuclei were found in cortical cells. The inner larger cells had small nuclei which were nearly always in the resting stage or were remaining quite unstained. Those cells also contained several large well-stained ellipsoidal or spherical granules and one or two somewhat faintly stained hexagonal bodies (Pl. V, Fig. 2).

b) Nuclear division in tetrasporangia. Tetrasporangia are cut off from the pericentral cells in the upper part of the branches. The connecting pit between the sporangium and the pericentral cells is usually stained dark. The sporangia are usually spherical or ovate but, rarely, elongated toward the base. The nucleus of young sporangia usually has a deeply stained nucleolus but, rarely, two. The successive stages of the first nuclear division observed are shown in Pl. V, Figs. 7–21. The spireme and synapsis stages could not be observed. In metaphase, nearly 20 gemini were counted (Figs. 13 & 14). Centrosome-like granules were rarely observed (Figs. 19 & 20), but no spindle was visible. After the second nuclear division was completed, the tetraporangium increased in size rapidly, cleavage furrows appeared at the periphery of the cell and gradually grew inward to divide the sporangium into four tetraspores. Then the chromatophores made their appearance in the periphery of each spore (Pl. VI, Figs. 11 & 12).

Laurencia papillosa *HARV.* and
Laurencia obtusa var. ***majuscula*** *HARV.*

In the present two species of *Laurencia*, the tetrasporic mateirals were fixed with two fixing fluids in the above list, Nos. 6 & 7, containing stock solution of chromic acid. These fluids gave excellent results in bringing forth good nuclear figures in both somatic cells and tetrasporangia.

a) Somatic division. Somatic nuclear divisions were observed in the cells of the trichoblasts at the apical depression of the thallus. The resting nucleus had a reticulum and a single, or rarely two, nucleoli in the center. In metaphase, the chromosomes were counted to be less than 40 in number. Spindle fibers could not be observed in either species, while the centrosome-like granules were sometimes clearly present in *L. papillosa* but not at all in *L. obtusa* var. *majuscula*.

b) Nuclear division in tetrasporangia. Young tetrasporangia sometimes contained several granules besides a central nucleus. These granules made it difficult to observe the

nucleus in the prophase stage of division but usually they disappeared by the time of metaphase. The resting nucleus of the sporangium usually contained one nucleolus, but sometimes two, or, rarely, in *L. papillosa* even three. The chromatin threads in early prophase very thin, faintly stained, and gathered at one side of the nuclear cavity. In *L. papillosa*, the nucleus containing two nucleoli was sometimes observed in synapsis to have two groups of chromatin threads, each of which was in contact with one of the nucleoli respectively (Pl. VII, Fig. 14). Nuclei in diakinesis were occasionally met with in *L. papillosa* (Pl. VII, Figs. 16 & 17); they were revealed by a careful observation to contain V- or dumbbell-shaped chromosomes. In metaphase of the first and the second nuclear divisions, a large centrosome-like body was always present at each pole in *L. papillosa* (Pl. VII, Figs. 18 & 19; Pl. XII, Figs. E-J) but not in *L. obtusa* var. *majuscula* (Pl. VII, Figs. 24–26). Spindle fibers were observed in the latter species (Fig. 26) but not in *L. papillosa*. Later stages in the development of tetrasporangia in the present two species are quite similar to those observed in the other two species described above.

SUMMARY

1. The present study deals with the mitosis in somatic cells and the meiosis in tetrasporangia of the following four species : *Polysiphonia japonica, Chondria crassicaulis, Laurencia paipllosa*, and *Laurencia obtusa* var. *majuscula*. The mitosis in spermatogenesis as well as in the development of procarps and gonimoblasts was also examined in *Polysiphonia japonica*.

2. The chromosome numbers of the four species here investigated have been established to be forty in the diploid nculeus and twenty in the haploid. These numbers are in accordance with those reported by Yamanouchi (1906), Kylin (1914, 1923) and Westbrook (1928) in other species of *Polysiphonia, Chondria*, and *Laurencia*, and also in two species of *Rhodomela*, but not with those reported by Austin (1956). (Cf. Table I).

3. In *Polysiphonia japonica* it was established that the nucleus of a spermatium goes down through the trichogyne and fuses in the carpogonium with the female gamete nucleus. It was also found that the gonimoblast initials do not come out directly from the auxiliary cell itself but from the large central cell formed by coalescence of the auxiliary cell with supporting cell, and also with the netural cells in the neighborhood of the procarp.

4. Centrosome-like bodies were observed in the nuclear division of the somatic cells in *Laurencia papillosa* and in the meiotic division of tetrasporangia in *Chondria crassicaulis* and *Laurencia papillosa*. However, they were absent in the division of the somatic cells in *Chondria crassicualis* and in the division of both somatic cells and reproductive cells in *Polysiphonia japonica* and *Laurenica obtusa* var. *majuscula*.

5. Spindle fibers were visible in the mitosis of the somatic cells in *Chondria crassicaulis*. They were also visible in the meiotic divisions of *Polysiphonia japonica* fixed with Abe's fluid and of *Laurencia obtsua* var. *majuscula*.

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EXPLANATION OF PLATES

PLATE I

Polysiphonia japonica Harv.

Figs. 1 & 2. Vertical sections through branch apices of a tetrasporophyte showing nuclear divisions in somatic cells and young tetrasporangia. × 480

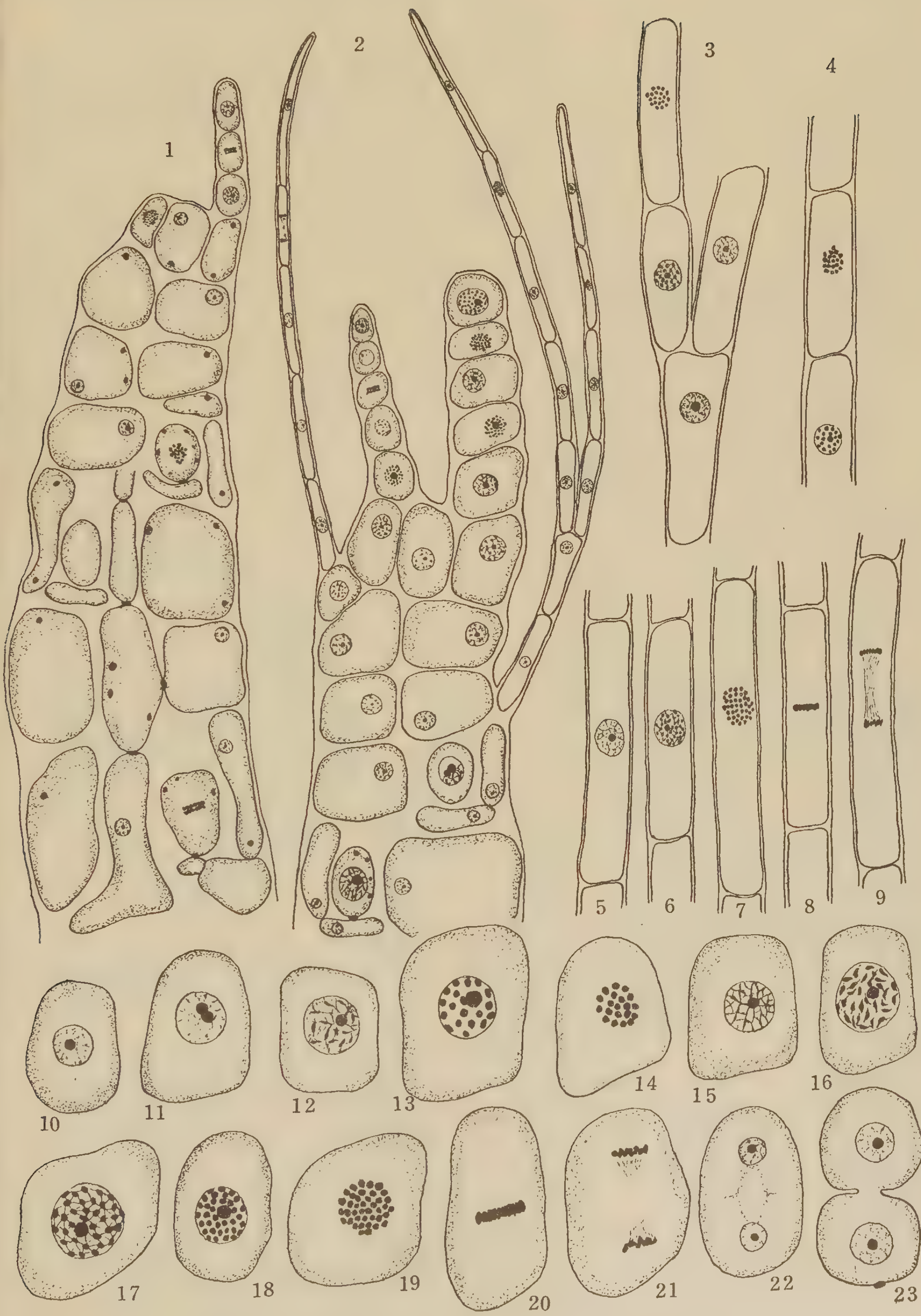
Fig. 3. Part of a hair on a female gametophyte showing somatic nuclear divisions. × 1440

Fig. 4. Part of a hair on a male gametophyte showing somatic nuclear divisions. × 1440

Figs. 5–9. Various stages of somatic nuclear division in cells of hairs on a tetrasporophyte. × 1440

Figs. 10–14. Various stages of somatic nuclear division in cells of a male gametophyte. × 1440

Figs. 15–23. Various stages of somatic nuclear and cell divisions in a tetrasporophyte. × 1440



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PLATE II

Polysiphonia japonica HARV.

Figs. 1–10. Various stages of the first nuclear division in tetrasporangia; 1 & 2, early prophase; 3 & 4, diakinesis stage; 5–8, metaphase; 9 & 10, anaphase. × 1260

Figs. 11–16. Various stages of the second nuclear division in tetrasporangia: 11 & 12, metaphase; 13, anaphase; 14–16, telophase. × 1260

Figs. 17–19. Various stages of cell division of tetrasporangia. × 1260

Fig. 20. Mature tetrasporangium. × 1260

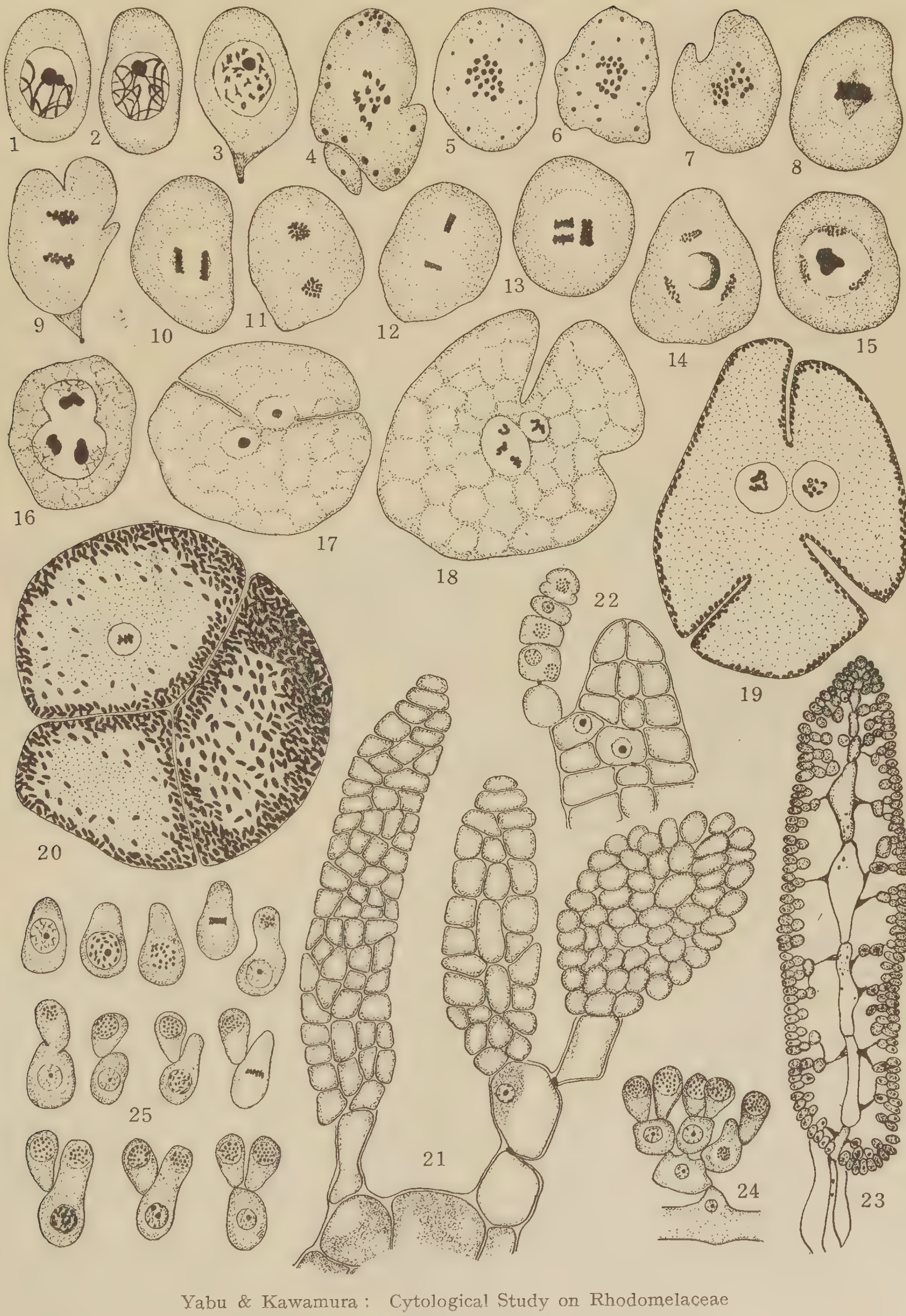
Fig. 21. Part of terminal portion of a male gametophyte showing young antheridia. × 840

Fig. 22. Part of thallus of a male gametophyte showing initial stage of development of an antheridium. × 840

Fig. 23. Vertical section of an antheridium. × 210

Fig. 24. Part of matured antheridium showing spermatangia and their mother cells. × 1260

Fig. 25. Various stages of nuclear division in spermatangium mother cells and in development of first and second spermatangia. × 1470



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PLATE III

Polysiphonia japonica HARV.

Earlier stages of development of female reproductive organs.

Fig. 1. Fertile trichoblalst showing fertile pericentral cell, which later gives rise to procarp, on suprabasal segment.

Fig. 2. Abnormality in the development of original cell of procarp.

Fig. 3. Metaphase of mitosis in a fertile pericentral cell on suprabasal segment of a trichoblast, showing 20 chromosomes.

Fig. 4. Young procarp.

Fig. 5. Young procarp with a sterile cell at the base of supporting cell.

Fig. 6. Young procarp with fully constructed carpogonial branch.

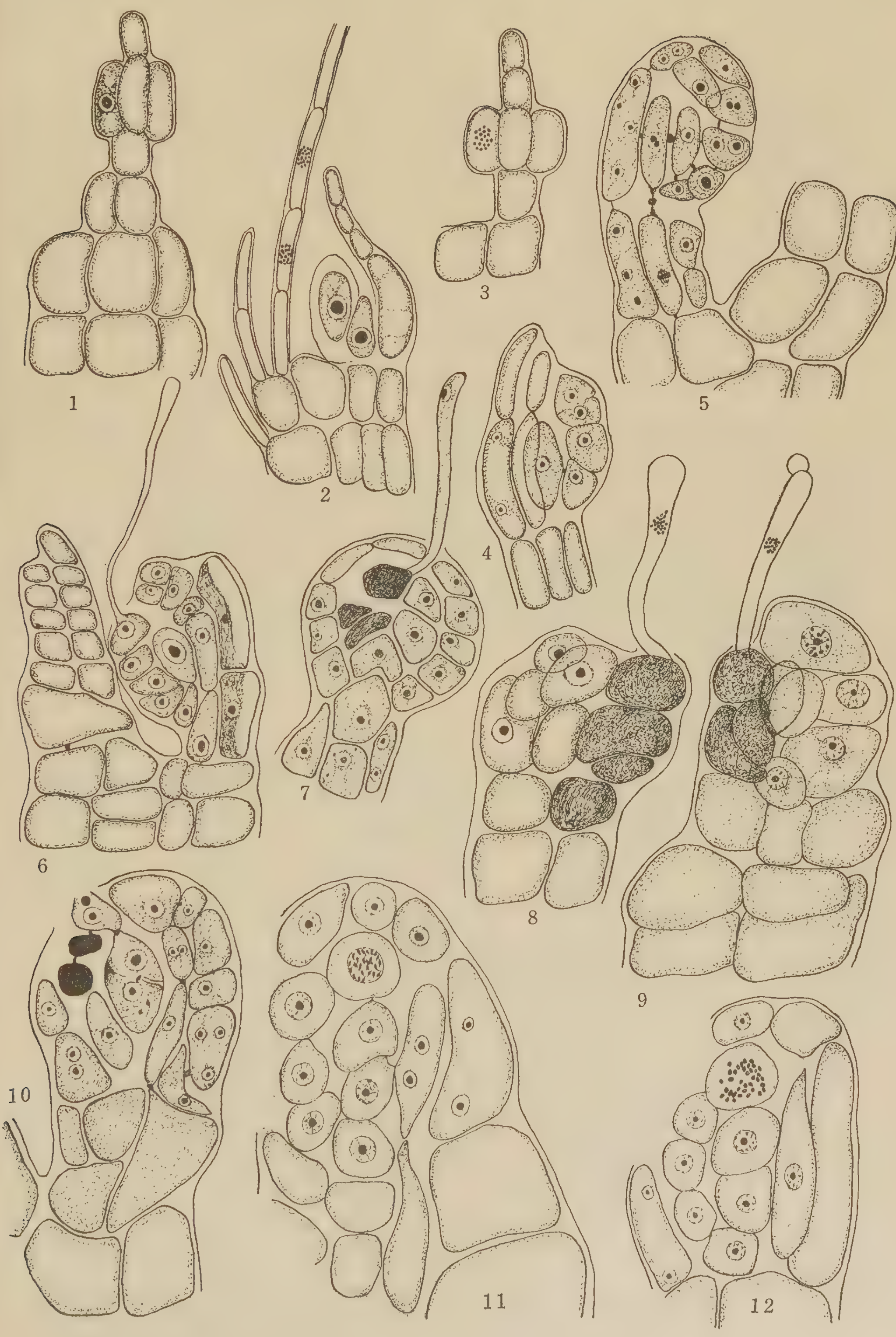
Figs. 7–9. Procarps showing passage of spermatium nucleus through trichogyne.

Fig. 10. Procarp at the time of fertilization and the formation of auxiliary cell.

Fig. 11. Procarp showing prophase of mitosis of fertilized nucleus in carpogonium.

Fig. 12. Procarp showing metaphase of mitosis of fertilized nucleus in carpogonium.

(Figs. 1–7, × 720; Figs. 8–12, × 960)



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PLATE IV

Polysiphonia japonica Harv.

Later stages of development of cystocarp.

Fig. 1. Procarp showing metaphase of mitosis of fertilized nucleus in carpogonium.

Fig. 2. Young cystocarp showing an auxiliary cell containing a diploid nucleus and showing fusion between the auxiliary cell and the supporting cell.

Fig. 3. Part of Fig. 2. enlarged to show details of a diploid nucleus.

Figs. 4 & 5. Resting diploid nucleus in the auxiliary cell which has fused with the supporting cell (Fig. 4) and also with adjacent cells (Fig. 5).

Figs. 6–10. Mitosis in fusion-cells derived from the fusion between the auxiliary cell and other cells.

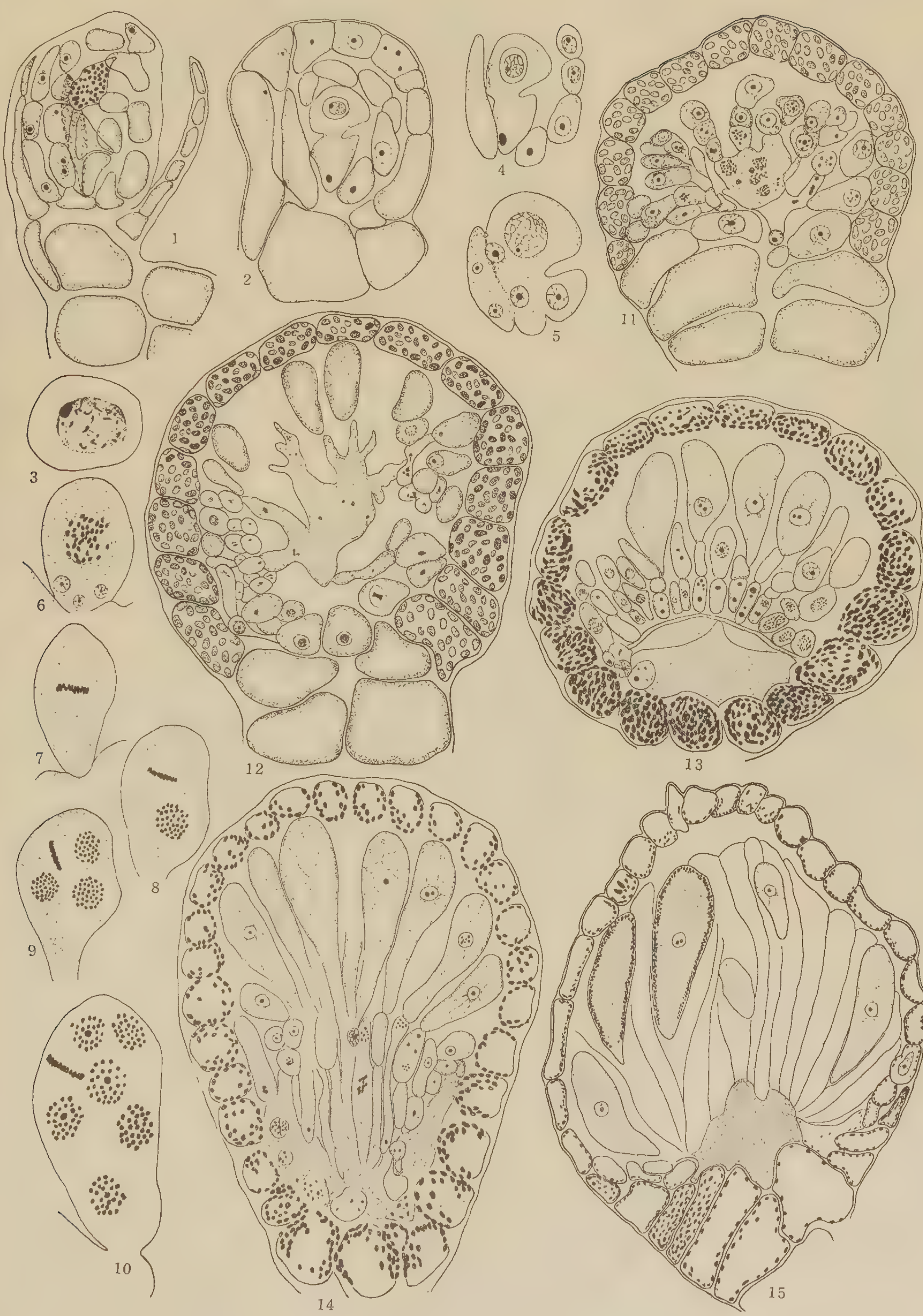
Fig. 11. Young cystocarp showing gonimoblast cells developed from the fusion cell.

Fig. 12. Young cystocarp.

Figs. 11–15. Successive stages of development of the cystocarp. In Fig. 15 there are shown walls of empty carposporangia remaining after discharge of carpospores.

(Figs. 1, 2 &, 4, ×600; Fig. 3, × ca. 2000; Figs. 5–10, × 750; Figs. 11–15, × 600)

PLATE IV



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PLATE V

Chondria crassicaulis Harv.

Fig. 1. Part of cross section through the upper part of a branch showing somatic cells with nuclei in various stages of mitosis and two tetrasporangia, each with a resting nucleus.

Fig. 2. Part of vertical section through the lower part of a branch showing nuclear divisions in the superficial multinucleate cells.

Figs. 3 & 4, Metaphase in somatic cells.

Figs. 5 & 6. Anaphase in somatic cells.

Figs. 7–12. Prophase of the first nuclear division in tetrasporangia.

Figs. 13–18. Metaphase of the first nuclear division in tetrasporangia.

Figs. 19 & 20. Side view of metaphase of the first nuclear division in tetrasporangia.

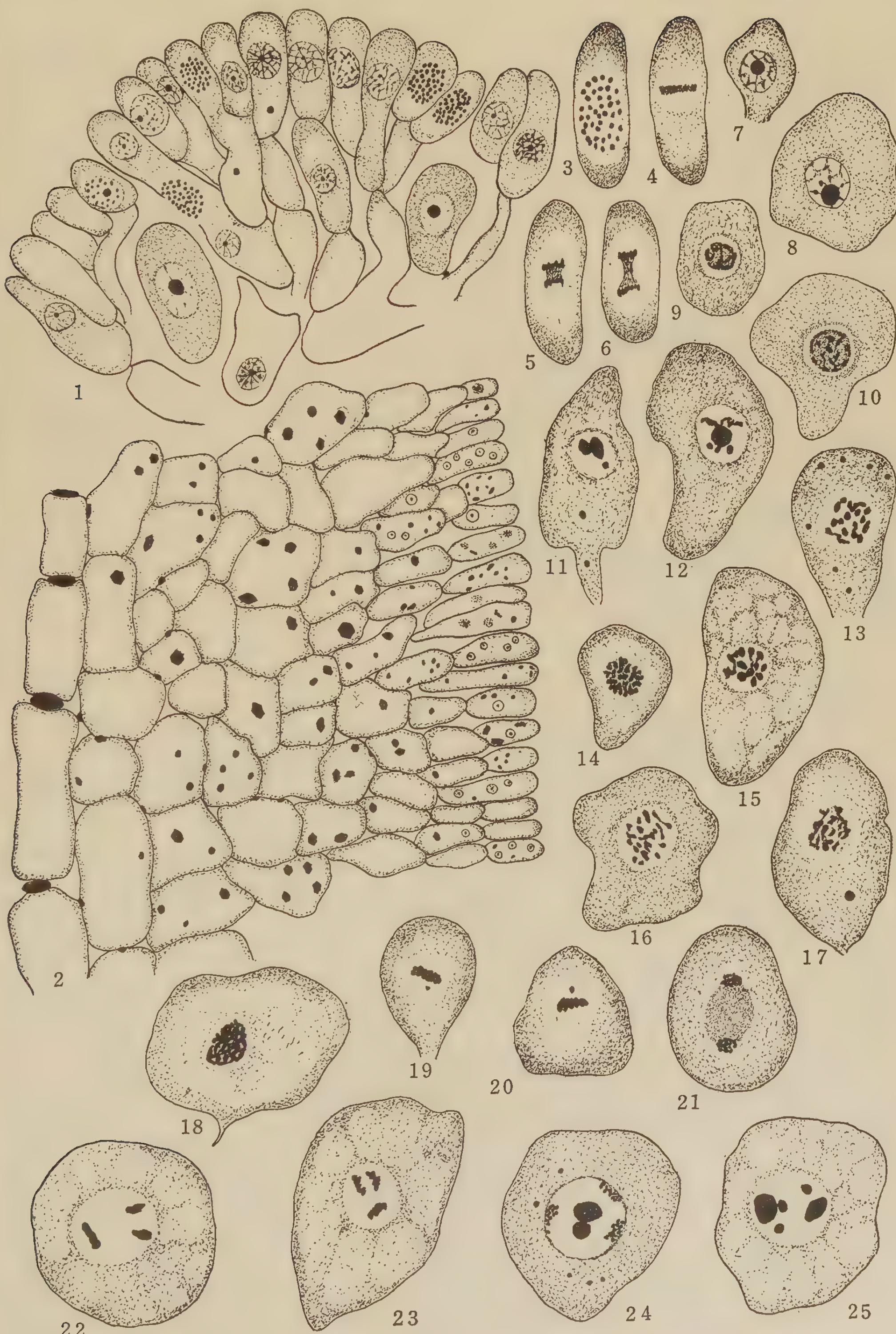
Fig. 21. Telophase of the first nuclear division in tetrasporangia.

Figs. 22 & 23. Metaphase of the second nuclear division in tetrasporangia.

Fig. 24. Telophase of the second nuclear division in a tetrasporangium.

Fig. 25. Later stage of the above, showing nucleolus-like granules.

(Fig. 1, × 1200; Fig. 2, × 640; Figs. 3–6, × 1680; Figs. 7–25, × 1200)



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PLATE VI

Chondria crassicaulis HARV.

Figs. 1–3. Tetrasporangia after meiosis of their nuclei has finished, showing nucleolus-like granules in the nuclear cavity.

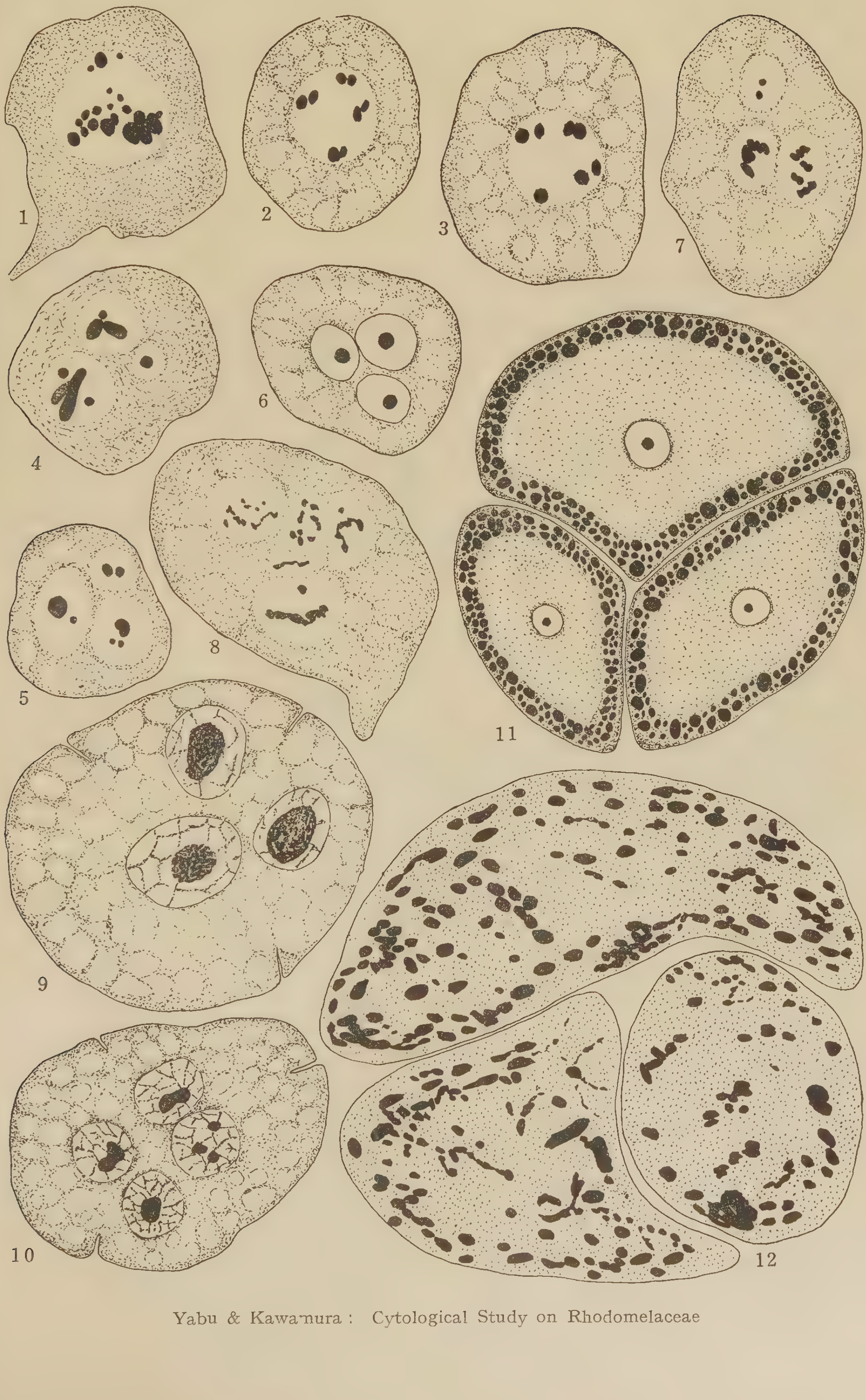
Figs. 4–8. Later stage than the above, showing four daughter nuclei.

Figs. 9 & 10. Tetrasporangia with four daughter nuclei; cleavage furrows appearing at periphery.

Fig. 11. Young tetraspores just separated by cleavage furrows.

Fig. 12. Mature tetraspores.

(Figs. 1–12, $\times 1200$)



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PLATE VII

Laurenica papillosa Harv.

Fig. 1. Longitudinal section through apical depression of thallus in tetrasporophyte, to show trichoblasts.

Figs. 2–6. Young trichoblasts showing mitotic figures in some of their cells : 2, apical cell containing two nucleoli; 3, late prophase; 4 & 5, metaphase; 6, side view of metaphase.

Figs. 11–22. Tetrasporangia, showing various stages of nuclear division and development of cleavage furrows : 14, synapsis, with two nucleoi, each of which is attached to chromatin threads; 16 & 17, diakinesis; 18, side view of metaphase in the first nuclear division; 19, side view of metaphase in the second nuclear division; 20–22, nucleolus-like granules in the nuclear cavity and cleavage furrows growing towards the center.

Laurencia obtusa var. *majuscula* Harv.

Figs. 7 & 10. Young trichoblasts showing late prophase of nucleus (Fig. 7) and side view of metaphase (Fig. 10).

Figs. 8 & 9. Metaphase in cells of a young trichoblast.

Figs. 23–30. Tetrasporangia showing various stages of nuclear division : 24, side view of metaphase in the first nuclear division; 26, anaphase in the second nuclear division; 27–30, later stages.

(Fig. 1, × 480; Figs. 2–10, × 1120; Figs. 11–13, 15–22 & 24–30, × 960; Figs. 14 & 23, × 1200)



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PLATE VIII

Polysiphonia japonica Harv.

Figs. A-C. Phototmicrographs of cross sections of tetrasporophyte, showing tetrasporangia each with dividing nucleus.

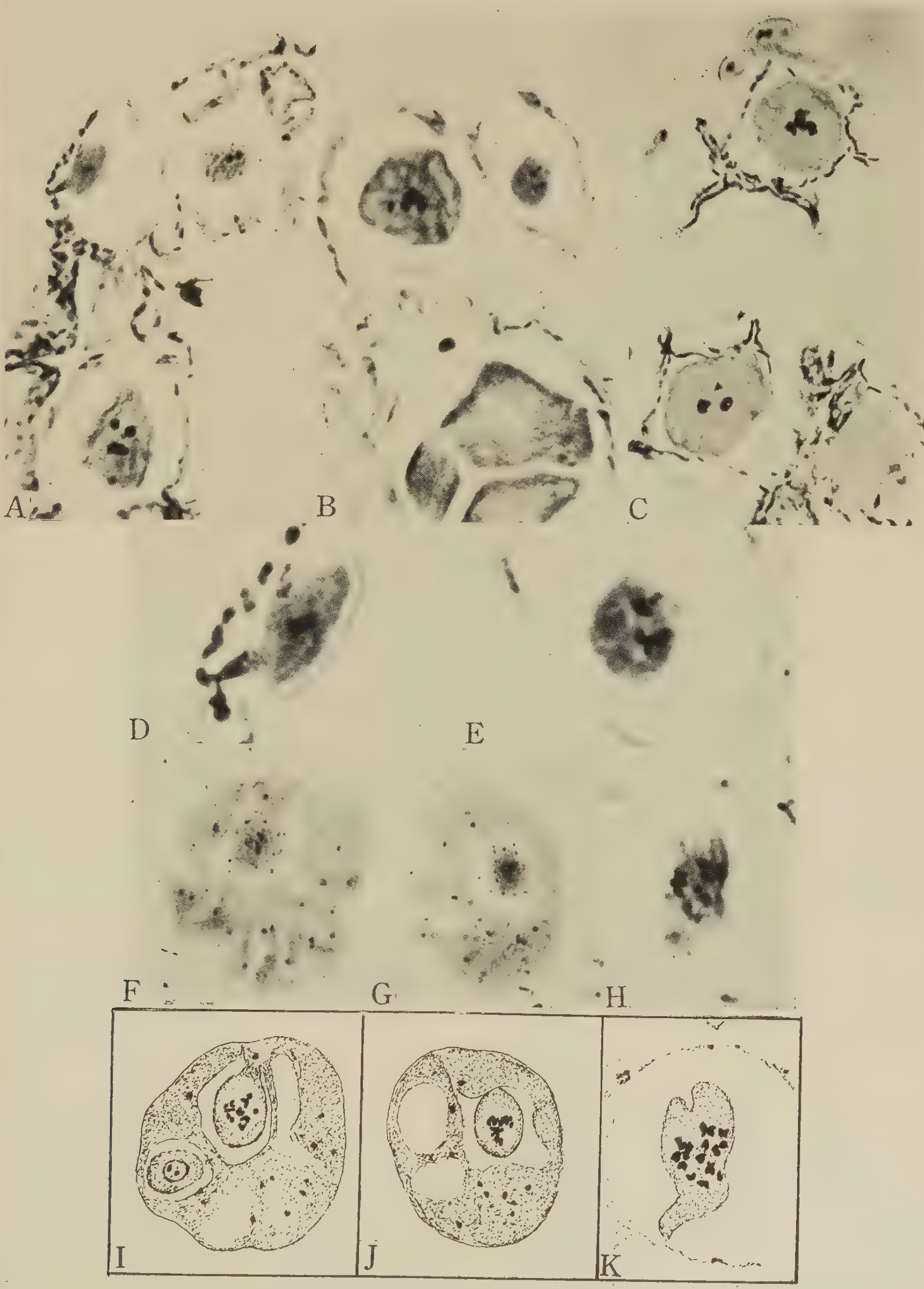
Fig. D. Part of Fig. A, enalrged, showing side view of metaphase of the first nuclear division in a sporangium.

Fig. E. Part of Fig. B, enlarged, showing anaphase of the first nuclear division in a sporangium.

Figs. F-H. Photomicrographs of cross sections of a tetrasporophyte, showing tetrasporangia with nuclei in metaphase of the first division (Figs. F & G) and in diakinesis (Fig. H).

Figs. I-K. Camera lucida drawings of the same sections as shown in Figs. F-H respectively.

(Figs. A-C, F, G, I & J, × 480,; Figs. H & K, × 960)



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PLATE IX

Polysiphonia japonica HARV.

Figs. A-C. Photomicrographs of feritle trichoblasts showing fertile pericentral cells (pc) and chromosomes (ch) at metaphase in cells of hairs.

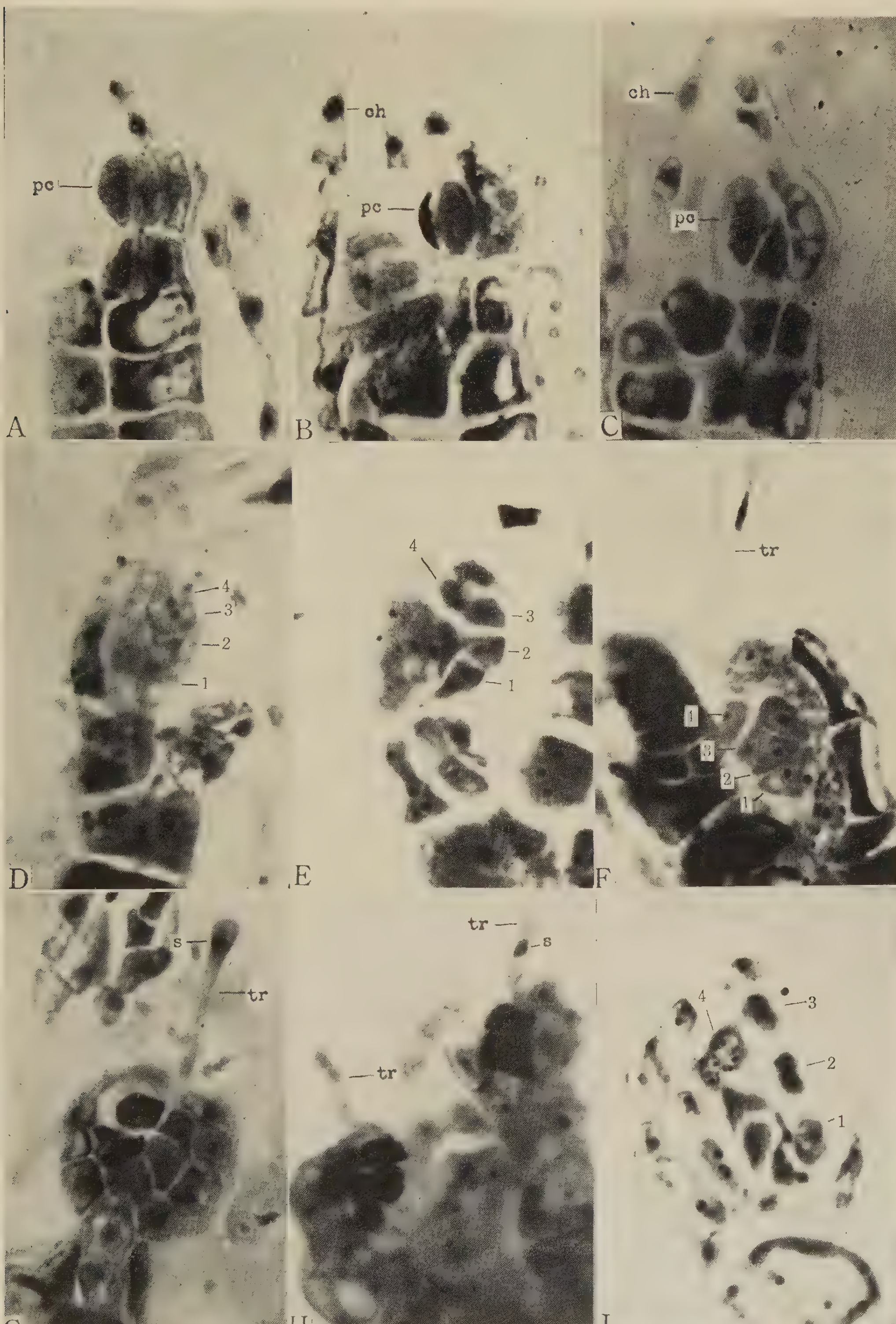
Figs. D & E. Photomicrographs of young carpogonial branches consisting of four cells (1–4). (*Cf.* Plate III, Fig. 4 and Fig. 5).

Fig. F. Photomicrograph of carpogonial branch consisting of four cells (1–4), of which the fourth cell (4) bears a trichogyne (tr). (*Cf.* Plate III, Fig. 6).

Figs. G & H. Photomicrographs of procarps showing passage of a spermatium nucleus (s) through the trichogyne (tr). (*Cf.* Plate III, Figs. 7–9).

Fig. I. Photomicrograph of vertical section of procarp, showing carpogonial branch (1–4), of which the fourth cell (4) has diploid nucleus in metaphase.

(Figs. A–G, × 720; Fig. H, × ca. 480; Fig. I, × ca. 960)



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PLATE X

Polysiphonia japonica HARV.

Fig. A. Photomicrograph of vertical section of procarp showing supproting cell (sc) which contains two nuclei and is just about to produce the auxiliary cell. (*Cf.* Plate III, Fig. 10).

Fig. B. Photomicrograph of vertical section of procarp showing auxiliary cell (ac) cut off upwardly from supporting cell. .

Fig. C. Photomicrograph of vertical section of procarp.

Fig. D. The same as Fig. C, the details of which were made clear by inking. Diploid nucleus in the fourth cell (4) of carpogonial branch is in later prophase; the nculeus of the supporting cell has divided into two and the supporting cell itself is in the way of division to produce the auxiliary cell (ac).

Fig. E. Photomicrograph of vertical section of procarp.

Fig. F. The same as Fig. E, the details of which were made clear by inking. Diploid nucleus in the fourth cell (4) of carpogonial branch is in metaphase; auxiliary cell (ac) is completely formed and is fused with carpogonium.

Fig. G. Photomicrograph of vertical section of young cystocarp with large fusion cell at the center in which is seen a side view of dividing nucleus (n) in metaphase.

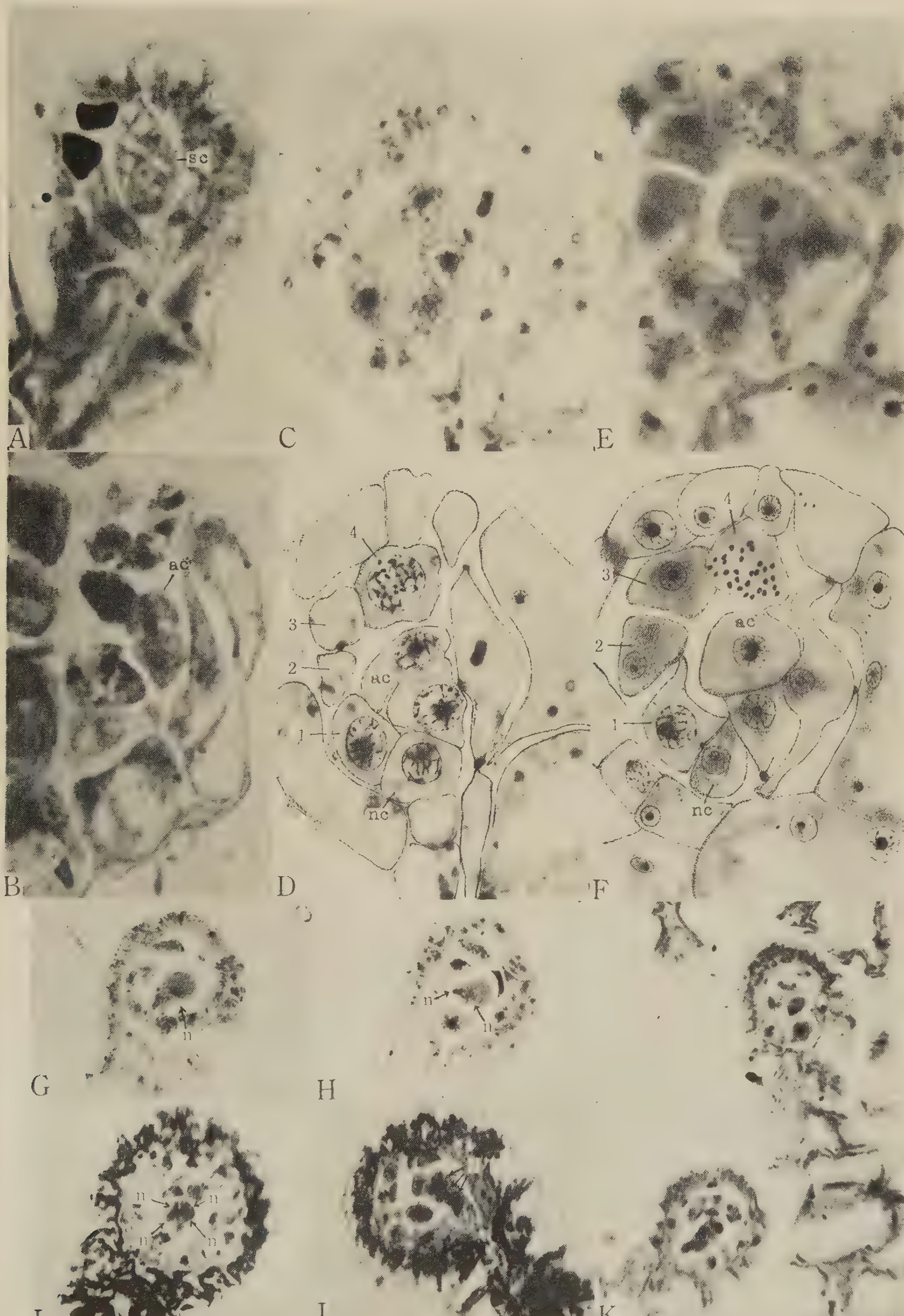
Fig. H. Photomicrograph of vertical section of young cystocarp, showing fusion cell with two daughter nuclei in metaphase (n).

Fig. I. Photomicrograph of vertical section of young cystocarp, showing fusion cell with four daughter nuclei in metaphase (n).

Fig. J. Photomicrograph of vertical section of young cystocarp, showing fusion cell with eight daughter nuclei (n).

Fig. K. Photomicrograph of vertical section of two young cystocarps.

(Figs. A-F, × 960; Figs. G-K, × 384).



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PLATE XI

Chondria crassicualis HARV.

Fig. A. Photmicrograph of cross section through tapical portion of thallus bearing hairs and branchlets.

Fig. B. Central portion of Fig. A, enlarged, showing a cell with two nuclei in metaphase.

Fig. C. Camera lucida drawing of the same cells as shown in Fig. B.

Fig. D. Photomicrograph of vertical section of a branchlet.

Fig. E. Part of Fig. D, enlarged, showing nucleus in metaphase.

Fig. F. Photomicrograph of cross section of thallus showing an abnormal tetrasporangium with elongated slender base.

Fig. G. Photomicrograph of cross section of thallus showing tetrasporangia.

Fig. H. Photomicrograph of cross section of thallus showing two tetrasporangia containing nucleus in metaphase of the first division.

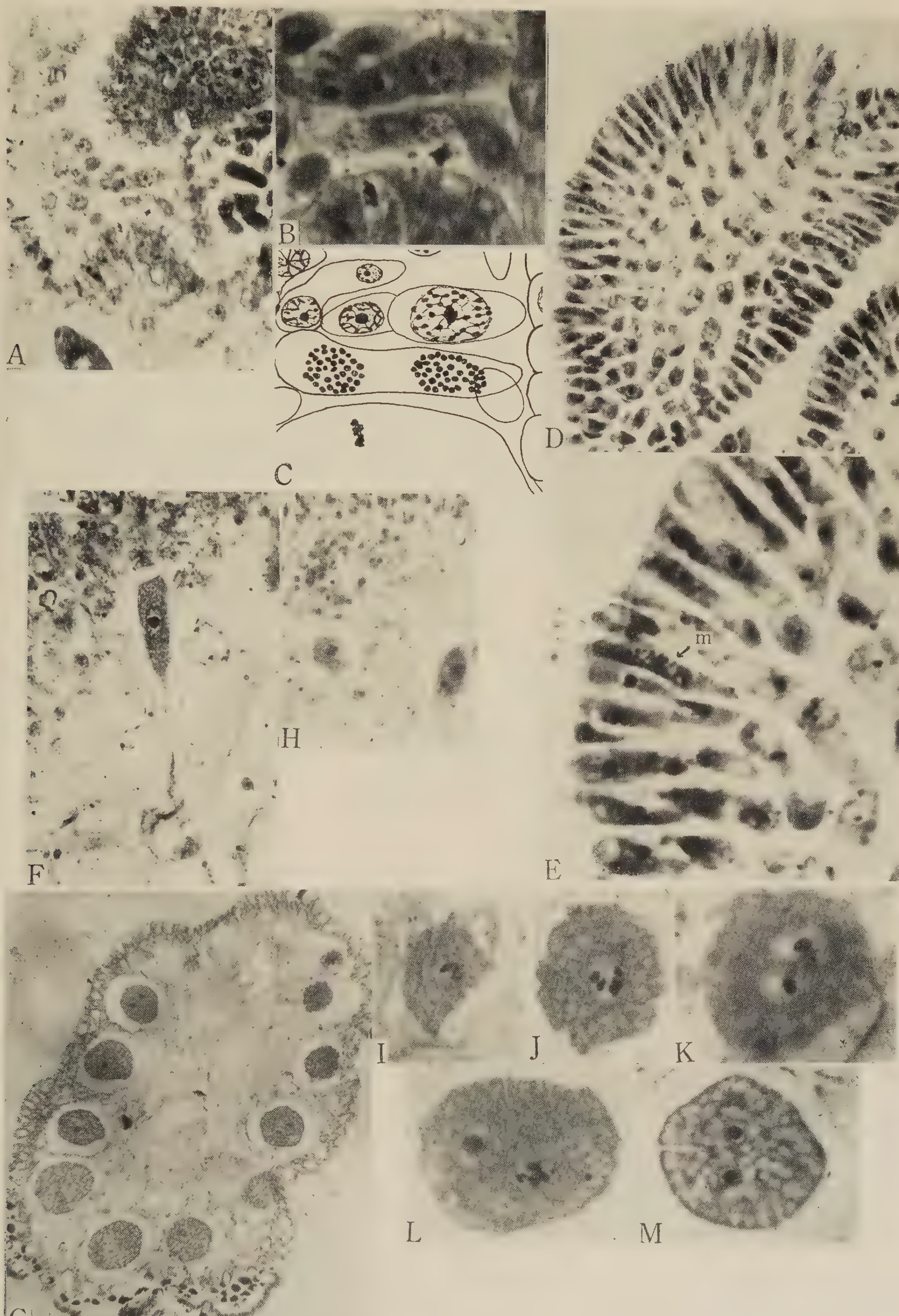
Fig. I. Photomicrograph of a sporangium with nucleus in metaphase of the first division.

Fig. J. Photmicrograph of a sporangium just after the meiosis was finished.

Figs. K-L. Photomicrographs of sporangia with separated daughter nuclei.

Fig. M. Photomicrograph of a sporangium with cleavage furrows growing towards its center.

(Figs, A, D, F, H, & M, × 3·4; Fig. G, × 64; Fig. I, × ca. 960; Figs. J, K, & L, × 720)



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PLATE XII

Laurenia papillosa HARV.

Fig. A. Photomicrograph of vertical section through apical portion of a stichidium showing young tetrasporangia and a cell with a side view of the dividing nucleus in metaphase.

Fig. B. Camera lucida drawing of the same section as shown in Fig. A.

Figs. E, G & I. Photomicrographs of sporangia whose nuclei are in metaphase of the first division.

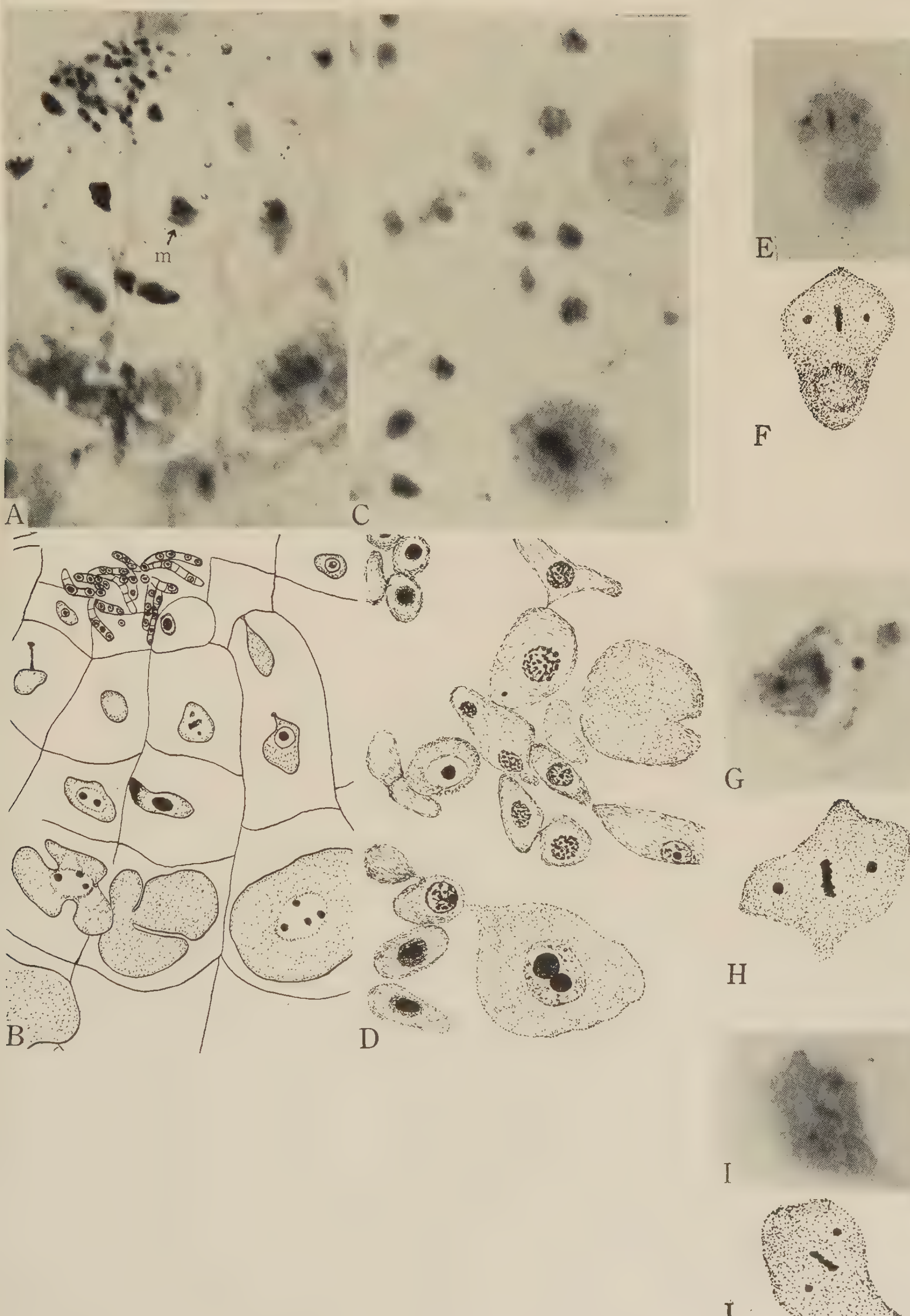
Figs. F, H & J. Camera lucida drawings of the same sporangia as above respectively.

Laurencia obtusa var. *majuscula* HARV.

Fig. C. Photomicrograph of collapsed section of thallus showing somatic cells with nuclei in porophase and a young tetrasporangium with two nucleoli in its nucleus.

Fig. D. Camera lucida drawing of the same section as shown in Fig. C.

(Figs. A & B, × 74; Figs. C, D, E-J, × ca. 1200).



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DEVICES OF SIMPLE PLANKTON APPARATUS

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CONTENTS

For sampling the plankton materials in lakes or at sea simple, sometimes hand-made, devices are usually employed, probably because not only of limitation of funds and facilities but also of difficutly in handling of either delicate or clumsy gear on the deck of a ship, especially when it is not well-equipped. It has been the writer's experience that finely constructed instruments which had been designed on land, work exactly in the air as expected, but not rarely do not do so when lowered into the depths of the sea. It is often true that simple gear works most efficiently. It would be better for one to think about sampling instruments while on board ship, perhaps even better in rough weather, than under the roomy and steady circumstances on land.

This report does not attempt to present designs of elaborate instruments of complicated mechanism. The samplers and a few pieces of laboratory apparatus described here are of simple construction, and can be made inexpensively. These devices have come into the writer's mind thinking about instruments for use in various fields of plankton studies which would be most practical in use as well as available for students of plankton under the present circumstances. Some of these designs have been actually constructed and tested at sea, but some models are only in the design stage. It is hoped that the ideas embodied in these devices may be improved by criticism and further actual use.

The writer is greatly indebted to Captain T. Fujii and crew members of the Training Ship "Oshoro Maru" for their help in the testing of instruments at sea. He also appreciates the help given by Dr. T. Kawamura and students of the University who were on board during the tests. Thanks are also due to Prof. Ki-ichiro Kobayashi for his kind advices given to the writer concerning the arrangement of figures in this report. The construction of models 17 and 18 by Rigosha Co., Ltd. free of charge is greatly appreciated.

I. DIVIDED VERTICAL HAUL WITH NET

At present the plankton net offers the simplest method for sampling the plankton, especially for medium-sized zooplankton. Inexactness of quantitative sampling with a net has been overcome to a certain degree through the use of an appropriate flow-meter.

In various studies on plankton, both biological and oceanographical, it is often desired that plankton samples be taken at various depths just as are the hydrographic measurements. During the vertical haul of a plankton net the contamination of materials from upper zones is prevented by closing the net after it has been hauled through the desired water layer. In most oceanic cruises, the hydrographic cast precedes the biological sampling, and it usually happens that there is no sufficient time to do the plankton sampling by making repeated vertical hauls from various depths. It is thus necessary to save time by sampling the plankton from various zones in a single haul. Hart's net[1] is one of the designs for this purpose.

Model 1. Twin net with semi-circular openings

(半月口双子ネット)

(Plate I, Fig. 17)

This is designed to sample the small-sized zooplankton from different vertical zones in a single haul. Two small nets of which the mouths are semi-circular are set on the metal frame. The frame is composed of top and bottom circular discs which are connected by four supporting rods with each other. Through the center of each disc the wire cable passes. Five or six such net assemblies will be prepared and each assembly is successively slid down along the wire cable while the cable is retrieved. As a result nets on the second assembly close the nets on first assembly, so that the latters no longer catch plankton. This design was already reported,[2] but has not yet been actually constructed.

Model 2. Continuous vertical sampler

(連続垂直採集器)

(Plate I, Fig. 18)

For finding out the relationship between abundance of small-sized plankton organisms and physical and chemical elements of the sea water through vertical range in detail, it is desirable to know by means of continuous sampling how plankton are distributed through the vertical column of water. The model 2 sampler is designed after Hardy's idea on the Continuous Plankton Recorder.[3–6]

A propeller mounted in the under part of the instrument is driven by the water current created by hauling of the instrument, and the revolution of the propeller, in turn, drives the spool inside with the result of continuous rolling of the silk gauze band wound on the spool. From the second spool which is located on the top left (in Fig. 18) another silk gauze band is drawn out with rolling. Plankton organisms entering from the top mouth are retained on the silk gauze when they pass across the rectangular path, and in the next moment, they are tightly gripped between two silk gauzes as they are transferred to the left (in Fig. 18) with the movement of the gauze. After hauling the instrument on deck the silk gauze band is unrolled to observe what and how many organisms have been taken at various depths through the vertical column of water. A small net of ordinary type which is mounted to the right side (in Fig. 18) collects the plankton through a column without dividing by zones. The quantity of water which had passed through the net will be known by means of a flow-meter mounted below the net.

Model 3. Twin net with bent lid

(曲蓋付双子ネット)

(Plate I, Fig. 19)

This model samples the plankton from any desired depth to the surface dividing them into two parts in a single haul. By lowering the messenger weight the bent lid closes the net on one side, while opening the net on the other side.

Model 4. Twin net for collecting in any two zones of water

(自由二層採集用双子ネット)

(Plate I, Fig. 20)

This model is slightly modified from model 3 in that the last sampling can be stopped beneath the surface. When the first meassenger hits the first trigger, one lid is dropped and the other lid is raised. The spring stretched between the lid and net frame at left side (in Fig. 20) is powerful enough to raise the other lid at right side (in Fig. 20) which is connected to the left lid by means of a metal chain. At the same time as this action the second trigger on the right lid comes into contact with the wire cable. The second messenger, which will be the same size as the first messenger, releases the clamp of the lid of the right net, and closes it.

Model 5. Four square net assembly

(角型四ネット組合せ)

(Plate I, Fig. 21)

This is constructed on the same principle as model 3, as it is essentially two model 3's put together. The first messenger closes the first net and at the same time it opens the second net. The second messenger, which has a size to cover the first messenger, closes the second net and opens the third net. The third messenger, which is the largest, closes the third net and opens the fourth net. To make the messenger hit against the triggers correctly, they are made to pass over a rigid rod which extends above the triggers.

Model 6. Double purse net

(二層巾着ネット)

(Plate II, Fig. 22)

This is designed for the same purpose of sampling as is model 3, but a modification of the design makes it possible to enlarge the size of the net. One of two purse nets is closed

and the other opened by the tripping weight which is released by impact of the messenger. A pretty large-sized net can be used.

Model 7. Double closing net

(二層閉鎖ネット)

(Plate II, Fig. 23)

The impact of the messenger releases a line by which the open net is throttled and at the same time a throttled net is opened. Construction is very simple and can be adopted to a net of considerable size. The throttling line must be strong enough to suspend the entire apparatus including a heavy weight.

II. HORIZONTAL OR OBLIQUE HAUL IN MID-WATER LEVELS

It is felt that for the study of vertical distribution of plankton, especially macroplankton, in detail, echelon vertical hauls are insufficient to yield useful data. The collection must be done by towing a series of nets horizontally or obliquely at different levels of water. The elaborate work performed by F. S. Russell on the problems of vertical distribution of macroplankton in the Plymouth area was carried out by such a method.[7]

Model 8. Horizontal net directly attached on the cable

(直結式水平ネット)

(Plate II, Figs. 24–26)

The simplest method of horizontal tow with a net through subsurface levels of water employs a net without bridles; the net is attached to the cable at both the upper and lower portions of the brass ring which encircles the mouth of the net. A series of such nets set on a single line is towed horizontally or obliquely through several desired zones. The line must be kept nearly vertical by means of a heavy weight attached to the lower end of the line. The contamination of samples from other levels of the water, which may be introduced when the nets are lowered or raised, is reduced to a great degree in this method. When the collections are limited to a shallow zone of water, say 30 meters deep or so, and when the water is calm, such a method seems to be very practical and particularly satisfactory.[8-11] The nets should rotate freely around the cable, otherwise the turning back of the twist of the cable line (often happens when a heavy weight is suspended) will result in deviation in the direction of the facing of the mouth of the net. In employing the design of Nishimura[12] (Fig. 26), it seems to be necessary to put a swivel at each connection point between the net ring and the cable.

If the nets are towed with too much speed, the towing cable line carrying the nets

will be suspended obliquely, thus the vertical cross section of the mouth of the net is much reduced. Such a condition occurs also when the ship is drifted by violent wind. This is often a problem at sea.

Model 9. Horizontal net with sliding ring

(滑り輪付水平ネット)

(Figs. 1–3, Plate III, Figs. 27–29)

To keep the mouth of such a net (model 8) vertical during horizontal tow regardless of the inclination of the towing cable, this model is designed, which automatically compensates

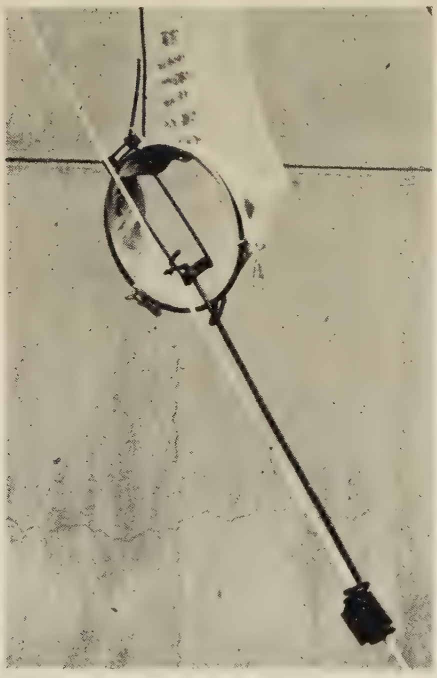


Fig. 1



Fig. 2



Fig. 3

Figs. 1-3. Horizontal net with sliding ring (model 9). 1. In lowering position. 2. In horizontal towing position. 3. In raising position.

for wire angle. The mouth ring of the net is mounted on a supporting rod which lies parallel to the cable, resting on a wire clamp beneath. This rod is able to rotate freely around the cable. The upper portion of the mouth ring is connected to the upper portion of the rod by means of paired hinged arms. The lower portion of the mouth ring has an eye by which the ring can slide up and down freely along the underhalf of the rod. When the cable is run out from deck, the mouth ring is raised until it lies parallel to the cable due to the current created by lowering the net into the depth, while in retrieving the towing cable back to deck the mouth ring is lowered until it becomes parallel to the cable. Thus, during the lowering and raising of the net, if the ship's movement (or current movement) is not large, contamination of materials from other zones will be much reduced compared with the case of the net having ordinary bridles. When towed horizontally the wire cable will be suspended obliquely, but the mouth of the net will face to the current at right angles, since the lower portion of the mouth ring slides along the rod until the net receives the largest resistance of water current.

The performance of this type of net was tested off south-western coast of Hokkaido in the Japan Sea on May 25 and August, 31, 1959. The first experiment was made by use of the procedure that the cable carrying a net was hauled immediately after it had run out 800 meters. The catch was almost negligible in this sampling, showing that the contamination of materials during the lowering and raising of the net had not occurred. In the second experiment nine nets were attached on the cable at 1 m, 50 m, 100 m, 200 m, 300 m, 400 m, 500 m, 700 m, and 900 m, and towed horizontally by drifting the ship for one hour. The action of the nets seemed to be satisfactory. The wire clamp of simple construction shown in Fig. 27 was proved to be easy to handle.

Model 10. Small horizontal net with rotary mouth ring

(口輪廻転式水平ネット)

(Plate III, Figs. 30–32)

A horizontal circular plate made of metal protrudes at right angles from a rod which is attached to the towing cable in parallel. The mouth ring of a small net (13 cm in diameter at the mouth) is hinged at both horizontal margins to the circular plate. There is a heavy balance weight which protrudes from the lower portion of the rod to the opposite side of the net. While the net is being lowered, the net end is forced to take an upward direction, resulting in the closing of the mouth of the net. During raising of the net, the net end is directed downward, the mouth of the net being closed. While the net is towed horizontally the mouth of the net faces in vertical to the current, leaving unclosed space. That space will be lessened when the towing cable is inclined, but still allows the plankton to enter into the net. The limitation of this type is in its small size of the net and, when towed horizontally, narrowness of the space between the mouth ring and circular plate.

Model 11. Triangular mid-water net

(三角中層ネット)

(Plate III, Fig. 33)

For sampling agile plankton animals from deep water it is necessary to employ a horizontal or oblique tow with a net of considerable size for a certain duration of time. The great distance through which the net has to pass in lowering and raising makes the sampling inaccurate because of contamination of materials which happens in these procedures.

Tucker's mid-water net[13] is a very fine design for collecting in a simple manner the fish larvae and other macroplankton from mid-layer regions of the sea with negligible contamination of the sample from the other levels. Model 11 is designed using his idea, but slightly modifying it. To keep the mouth of Tucker's mid-water net in a vertical position while it is being towed horizontally, a very heavy weight must be hung under the mouth of the net. Construction of a net having reversed triangular mouth as in Fig. 33 will decrease the resistance of the underside of the net to the water current which makes it easy to keep the mouth of the net near vertical position. The upper side of the triangle at the mouth of the net is made of a strong steel rod or pipe, and the other two sides are made of chains. Bridles connected to the upper rod are also made of chains. This construction makes the net easy to handle on deck. A long rod (preferably made of bamboo) is attached between the center of the steel rod and net end to prevent the body of the net from entangling with the towing cable during lowering.

This type of net was constructed and used in September 1959 during the carrying on of the program for deep sea research on the Japan Trench using the "Ryofu Maru" of the Meteorological Agency. According to the personal communication of Mr. Y. Komaki who was in charge of operation of the plankton sampling on board this ship, a big tapered wire carrying a triangular net was run out as long as 6000 meters at a station where the sea depth is 6500 meters, and again as long as 3764 meters at another station where the depth is 3800 meters. In both cases the sampling was successful. The net employed was of smaller size than that shown in Fig. 33. The triangular mouth of the net was 150 cm long at one side. The net was made of coarse nylon cloth in the anterior 300 cm and nylon bolting cloth NG 54 in the posterior 300 cm. A lead weight of 50 kg was suspended beneath the net by connection with 150 cm steel chain. In towing at 0.5–1 knots it was felt that a more heavy weight is needed to keep the mouth of the net in vertical position.

III. MECHANISM OF OPENING AND RECLOSING OF THE NET

To catch the zooplankton by horizontal or oblique tow only in the mid-water regions, the net must be closed until it reaches a desired depth, and after having been towed for a certain distance in open condition, it must be closed again before being hauled on deck.

There have been many devices constructed for this purpose.[14-28] It is a rather difficult thing to make reliable releasing of the clamps on the mechanism for opening and reclosing the net at a great depth by means of sliding messenger weight. The workers on deck have no way to know whether the first and second clamps on the mechanism are released properly or not. For this reason Van Cleve[30] constructed an electrical closing mechanism to overcome the inexactness of releasing of an ordinary mechanism, but it has not been widely used because of limitation due to the necessity of the electrical cord. It seems that at present Leavitt's[23,29] and Discovery[17] types of double releasing mechanisms are most widely used for deep oblique tow with large nets. In usual double releasing mechanism the second messenger is constructed of larger size than the first messenger to overlap the latter. Harvey's double releasing gear[20] is equipped with a spring system of appropriate strength by means of which two equal-sized messenger weights can be used.

Model 12. Double releasing mechanism with dissolving substance

(溶解式二重放索器)

(Figs. 4–6, Plate IV, Figs. 34–40)

This model is composed of a cylinder and follower piston. Some kind of dissolving substance (hard candy balls can be used) is put inside of the cylinder. After a certain length of time which is sufficiently long for the closed net to be positioned in the desired depth, the partial dissolution of the candy balls permits the first releasing arm to open which results in the opening of the net. Further dissolution of the balls permits the second arm to open which results in the reclosing of the net. Thus the open net is towed in the mid-water region for the time from the first releasing to the second releasing. In the test at sea this mechanism worked as expected, although it was difficult to anticipate the exact time required for dissolution of the balls. A cable connector shown in Fig. 39 makes easier the attaching and detaching the mechanism from the wire cable.

Model 13. Double releasing mechanism with long rotary neck

(長首式二重放索器)

(Plate V, Figs. 41–42)

The design of model 13 is different from those which have been hitherto reported. In this model the second trigger is located apart from the wire cable at first, but when the first messenger releases the first clamp, the second trigger comes into contact with wire cable. The first and second messengers can be constructed of the same size and weight. This mechnaism is only in the design stage.



Fig. 4



Fig. 5



Fig. 6

Figs. 4–6. Double releasing mechanism with dissolving substance (model 12). 4. Before releasing first and second arms (net is closed). 5. First arm is released (net is opened). 6. Second arm is released (net is reclosed).

Model 14. Double releasing mechanism with horizontal bar

(横棒式二重放索器)

(Fig. 7, Plate V, Figs. 43–46)

This model is designed for use on a wire of considerable thickness, e.g., 23 mm (in diameter) trawl warp or oceanographic big tapered wire in which the thickness is gradually lessened toward the end of the cable. The jar-shaped second messenger when lowered, overlaps the first messenger together with the upper part of the body of the instrument. The underside of the second messenger pushes down the horizontal bar (second trigger) which extends at both sides of the instrument. The lowering of the horizontal bar results in release of the second clamp. The first messenger never touches the second trigger in any way, so that there is no possibility of releasing the second clamp when the first messenger is lowered. The center hole of both messenger weights will be made to be large enough to pass over the wire cable even if there should be an inconstancy in thickness.



Fig. 7. Double releasing mechanism with horizontal bar (model 14).

IV. UNDERWAY SAMPLER

Many types of underway plankton samplers have been hitherto reported, such as Hensen's basket net and horizontal cylinder net;[31-34] Borgert's tow net;[31-33] Apstein's plankton tube;[31-33] Monti's collector;[31] Hardy's plankton indicator[35-37] and continuous plankton recorder;[3-6] Sheard's high-speed tow net;[38-39] Tamura's sampler;[40] Tester & Stevenson's metal net;[41] Arnold's high-speed plankton sampler;[42] Gehringer's all-metal plankton sampler;[43] Motoda's high-speed successive plankton sampler[44] and underway plankton catcher;[45] Ahlstrom, Isaacs, Thrailkill & Kidd's high-speed plankton sampler;[46] Miller's high-speed sampler;[47] etc. The small type Hardy Plankton Indicator[37] having a small conical net is apparently most practical to check the plankton distribution simply at any one position in the ocean while the ship is underway. The Hardy Continuous Plankton Recorder[3-6] is an ingenious and excellent sampler for use of continuous observation on the distribution of plankton over wide areas of the ocean. It has been extensively used around

Great Britain and also to a certain extent in the western Atlantic off the northern part of the United States. All-metal Gulf-III sampler[43] (so-called tin-tow net) is being used in the United States and also in England. The big size of this sampler (in the original model, the case is 224 cm long, 49 cm in diameter of cylindrical part, and 41 cm in diameter of mouth opening) makes it most efficient for collecting the agile large plankton animals which play an important role in the fisheries problem.

Model 15. High-speed tow net with heavy head

(頭棒付高速ネット)

(Fig. 8, Plate VI, Figs. 47–49)

There is a very clever device of high-speed tow net.[38,39] In this device the tail end of a net of ordinary conical shape is drawn up to the center of the mouth through the inside of the net. When towed at high speed the filtering capactiy is greatly increased in this folded net compared with the ordinarily stretched long net, and, in addition, the frontal



Fig. 8. High-speed tow net with heavy head (model 15).

wave occurring in front of the mouth of the net is much reduced. This is an adoption of Bernoulli's principle. The sampling with this type of net was reported to be successful at 5 knots (61 cm mouth diameter)[38] and 7 knots (46 cm mouth diameter).[39]

The model 15 sampler is designed under the same principle. Instead of ordinary bridles a heavy metal rod extends from the center of the short metal cylinder which is followed by the net. (This part was made by reconstruction of the underway plankton catcher III[45]). The area of mouth opening excluding the part of central rod measures 44 cm². The rod and mouth cylinder altogether weigh 2.4 kg. Experiment at sea proved that by running out of Manila rope of 18.2 mm diameter far long as 25 meters length from deck to the sampler, the model 15 equipped with a net of bolting silk GG 40, 0.47 mm mesh size, could be towed at 8.5 knots through the surface water without jumping on the sea surface. The strain was 21 kg. When towed with a depressor, the anlge of rope was 75 degrees and the strain was more than 100 kg.

Model 16. Simple underway plankton catcher IV & V

(簡易航走採集器 IV 型及V型)

(Figs. 9, 10, Plate VI, Figs. 50–54)

The numbering of this model succeeds after the numbers of the previously reported three models of underway plankton catcher.[45] In the original design of underway plankton catcher I and II, the head piece is conical shape with two side openings, and the towing cable is attached at the tip of the head. This construction is intended to reduce the strain when the instrument is towed at high speed for the sake of making manual handling easier. However, it was felt that the instrument, when towed, was forced to rotate, resulting, in turn, in twisting of the towing cable, sometimes resulting in its breaking. In the model 16 the place of attachment of towing cable is changed to the upper side of the anterior portion of the head piece, like in the Hardy Plankton Indicator, and a wing underside of the posterior portion of the cylinder is removed. The design is recommended for its most simple and inexpensive construction as well as for easy handling in setting and removing the net inside of the cylinder. In catcher IV (Figs. 9, 10, Pl. VI, Figs. 50–52) the diameter is decided to fit the size of the Rigosha small type flow-meter[48] which is set inside of the inner cylinder. The iron cast head piece is pierced by a hole, 3 cm in diameter, which allows the water to pass through. Inside the cylinder a smaller inner cylinder is inserted which supports a small conical net (made of bolting cloth GG 56, 0.33 mm mesh size) at the anterior end and contains a flow-meter in the posterior portion. This inner cylinder is fixed by means of a pin which passes transversely through the outer cylinder near the end opening, so that attaching and detaching of the inner cylinder is very easy. The whole assemblage weighs 4.4 kg. This instrument can be towed at high speed through the surface water without jumping on the sea surface. The tow with a single sampler at any desired depth or simultaneous tow with several samplers through several zones can be made by the methods illustrated in Figs. 53 and 54. The test tow using a Manila rope (18.2 mm in diameter) of 25 meters length from deck to the sampler showed that, in the surface tow at 7.5 knots, the strain was 12 kg and that in the subsurface tow by means of a depressor at the same speed the strain increased to more than 100 kg. In both



Fig. 9. Simple underway plankton catcher IV (model 16). A depressor is laid on deck.

cases the sampler was very stable.

If the ship is large enough to handle the sampler on deck, and a powerful winch is equipped, the larger size of sampler, better. In catcher V (Fig. 10) the case including the head piece is 100 cm long and 15 cm in diameter. The iron cast head piece which itself is 14 cm long is pierced by a water tunnel, 7 cm in diameter. The tripod frame which is inserted inside the case from the end opening, carries a net and a flow-meter. The whole assemblage weighs 15 kg.

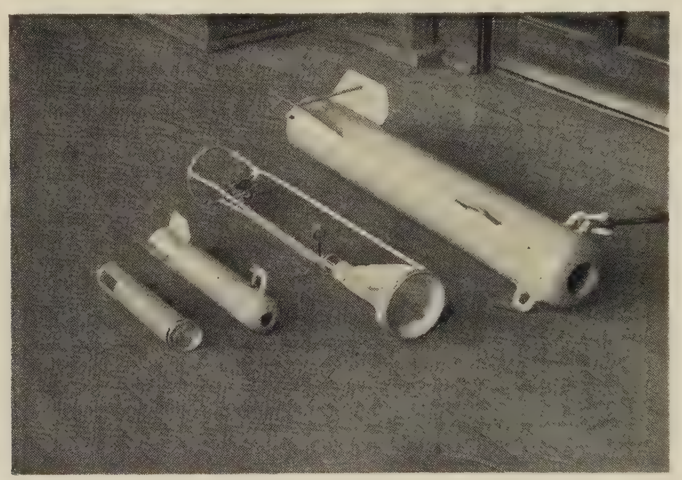


Fig. 10. Simple underway plankton catcher IV (left) and V (right) (model 16). Inner cylinder of catcher IV and inner tripod supporter of catcher V which carry a net and a flow-meter are drawn out from the case.

Model 17. Simple underway plankton catcher VI with outside frame

(外枠付簡易航走採集器 VI 型)

(Plate VI, Figs. 55–57)

A rotary rectangular frame is attached outside the cylindrical case to which a towing cable is connected at its upper ring and a depressor cable at its lower ring. This permits the sampler to keep horizontal position irrespective of inclination of the towing cable. The whole assemblage weighs 9.4 kg. In the test at sea this model was towed at 8 knots using a Manila rope (18.2 mm in diameter). The instrument ran stably and the strain was more than 100 kg.

Model 18. Multiple net underway sampler

(多網式航走採集器)

(Figs. 11, 12, Plate VII, Figs. 58-60)

The instrument is mounted in a long rectangular case having both ends tapering. Ten small nets, 3.7 cm in diameter, are arranged on a narrow metal plate which can be easily removed from the inside of the case. This plate pushes up a flexible metal band which moves forward with the rotation of reels. Anterior opening of the case which admits the water is 2.5 cm square. The water entering this opening passes along the water tunnel upside, and reaches the intake cap which is fixed on the flexible metal band, then turns into net. The instrument is towed at high speed by means of a thick cable, and, in addition,

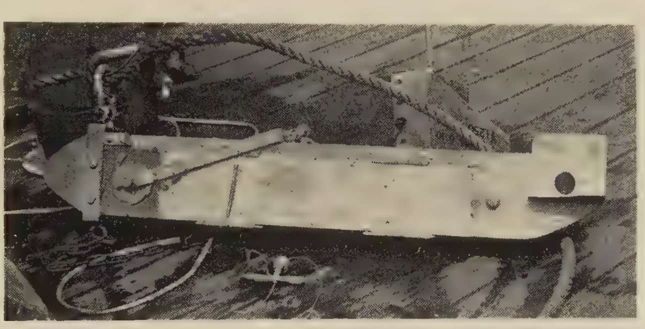


Fig. 11

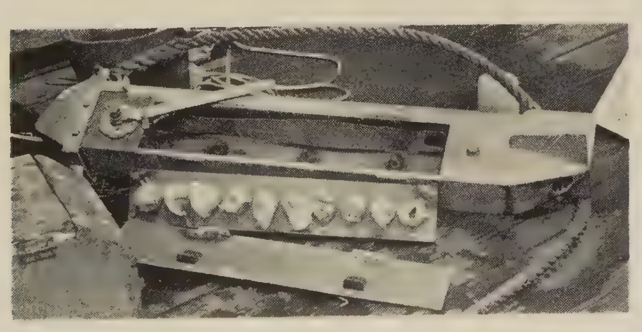


Fig. 12

Figs. 11-12. Multiple net underway sampler (model 18). 11. Outside view. 12. Narrow metal plate carrying ten small nets is removed from the case.

an accessory line (also thick cable) is connected from the upper ring of the rectangular frame to deck, but is not tensioned. By pulling the accessory line from deck (actually by slacking the main towing cable), the rectangular frame is pulled forward with the result that the rotation of the anterior reel is caused sufficient to shift the intake cap on the

flexible metal band from one net to another net. Successive pulling the accessory line (actually successive slacking of the towing cable and accessory line alternately) at every desired distance of the tow enables the sampling of the plankton into different nets successively. The tow cannot be adopted for a long distance beyond the time of decaying of the organisms sampled. The whole assemblage weighs 14.1 kg. In the test tow carried out at sea, it was found that more tight contact between the metal band and the narrow plate carrying the nets is needed.

Model 19. Multiple net underway sampler with storing tank

(貯蔵タンク付多網式航走採集器)

(Plate VII, Figs. 61–65)

In this model instead of shifting of the location of the water intake cap in model 18, the nets themselves move with the rotation of the reel. Fitfteen small conical nets, 5 cm in diameter at the mouth and 5 cm deep, are arranged on the flexible metal band which is rolled between fore- and rear-reels. The net is turned over inside out at first, but when it comes under the inner rectangular opening of the water tunnel, which is 5 cm wide and 8 cm long, i.e., longer than the diameter of the net, it is forced back inside in due to water current. This device is for the purpose of preventing contamination of samples in the net before it is connected to the water tunnel. The net, after being connected to the water tunnel for desired duration of time, is pushed into the formalin tank when the accessory line is pulled. The net passes through the narrow space between the upper and lower rubber flaps at the entrance to the formalin tank. If one net samples the plankton for 20 miles, the total collection will cover 300 miles. Pulling system for shifting the net can be replaced by the propeller and gear system if automatic successive sampling is desired. This instrument is not yet actually constructed.

V. PLANKTON SUBSAMPLING APPARATUS

When the sample is too big, it is necessary to take only a known fraction of the sample for counting the number of individuals in it. Several types of plankton sample fractioning apparatus have been devised. They are Hensen's shaking flask with pestle pipette ;[31,32] Gibbon's cylinder with multiple septa ;[49] Harvey's stirring cylinder and dip stick ;[22] Wiborg's whirling vessel ;[50] Kott's modified whirling vessel ;[51] Folsom plankton sample splitter ;[52] etc.

Model 20. Plankton sample splitting box

(プランクトン標本分割箱)

(Figs. 13–15, Plate VIII, Figs. 66–68)

This model is a plastic box having a longitudinal septum inside. The septum is set on the center line extending through the half of the box and this is succeeded by a low ridge, 5 mm in height, on the bottom of the other half. The upper plate covers the half of the box on the portion of septum except for a rectangular opening at one corner. The method of splitting is: 1) Tilt the box to the open portion (portion without cover) on the edge of table, pushing the base piece of the box against the edge of the table; 2) Stir sample with a thin stick; 3) Tilt the box to the opposite side. Sample is split into two subsamples by the septum; 4) Turn the box over to pour out one subsample into a beaker through the open corner of the cover. The other subsample does not flow out because prevented by the cover; 5) Return the box to normal position; 6) Tilt the box to the covered portion and put in rinse water with a pipette to wash down the adhering organisms to the covered portion. The low ridge on the bottom plate prevents the mixing of organisms in two split parts; 7) Turn the box over to pour out the washed organisms into the same beaker; 8) Repeat the procedure of steps 5–7. The splitting box of size given in Figs. 66 and 67 can be used for a sample as large as 1500 cc.

Model 21. Plankton sample splitting cylinder

(プランクトン標本分割円筒)

(Plate VIII, Figs. 69–71)

A plastic cylinder, 9 cm in outer diameter and 15 cm in height, is fixed on a square plastic base plate, 10 cm square. In the cylinder there is a perpendicular septum dividing the interior equally. This perpendicular septum is in parallel to two sides of the square base plate. A quarter of the top of the cylinder is covered by a plate. In tilting the cylinder septum up, the sample is stirred in the lower portion of the cylinder, and then the cylinder is tilted to opposite side. The sample is thus split into two halves by the septum. By tilting the cylinder farther the one of the split samples is poured out from the cylinder into a beaker. Adhering specimens should be rinsed down by adding water with a pipette. This splitter is used for a small sample, less than 150 cc of water containing the organisms.

VI. PLANKTON SAMPLE FILTERING APPARATUS

For rapid and accurate measurement of displacement volume of a plankton sample, various filtering apparatuses have been devised.[53-57] In processing the samples brought from an ocean cruise it is often necessary to deal with hundreds of samples for such processing.

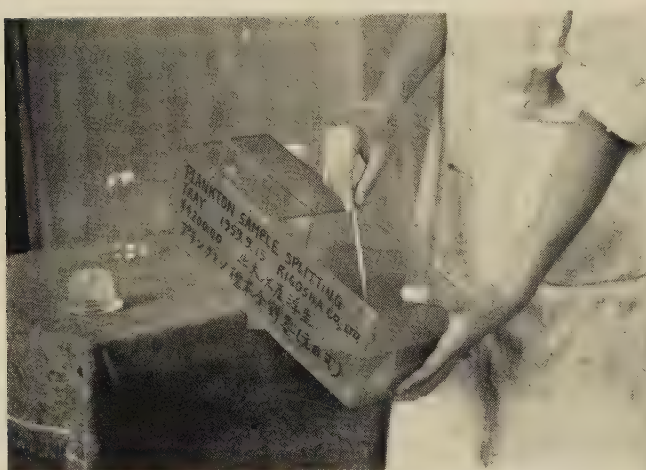


Fig. 13

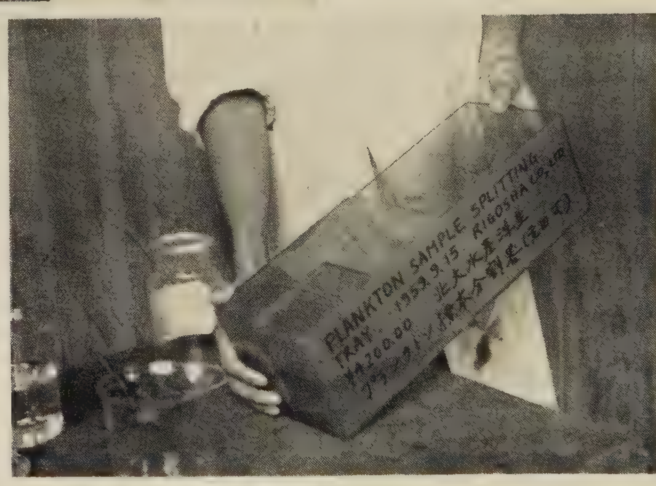


Fig. 14



Fig. 15

Figs. 13–15. Plankton sample splitting box (model 20). 13. The box is tilted to the open portion, and the sample is stirred with a thin stick. 14. The box is tilted to the covered portion. Sample is split into two subsamples by the septum. 15. The box is turned over, and one subsample is poured into the beaker.

Some of them are fairly big, and sometimes contain many phytoplankton which are not easily filtered by ordinary gravity filtering method. It is hoped to devise a tool for such measurement by means of aspiration and adequate filtering apparatus.

Model 22. Plankton sample aspiration filtering apparatus

(プランクトン標本吸引濾過装置)

(Fig. 16, Plate VIII, Figs. 72, 73)

This apparatus is composed of upper and lower funnels (made of metal or plastic); cylindrical aspiration chamber (made of transparent plastic); and a receptor of filtered water (glassware). The assemblage of these parts is kept in place on a metal tripod supporter (52 cm in height). Beneath the receptor is placed a graduated glass cylinder, 200 cc in capacity. A glass filter or paper filter or bolting cloth is used for filtration according to the purpose of the study. The filter is tightly gripped between the upper and lower funnels by means of three clips at the margin of the rims of the funnels. If the paper filter is used, glass filter or other appropriate sieve should be put under the paper filter. A narrow metal tube is fixed into the aspiration chamber from the thick rubber plate underside, and a rubber tube is connected from the bottom of this metal tube to the electromotive aspirator or to the vacuum aspirator or to the simple aspirating apparatus employed by Hart.[1)]

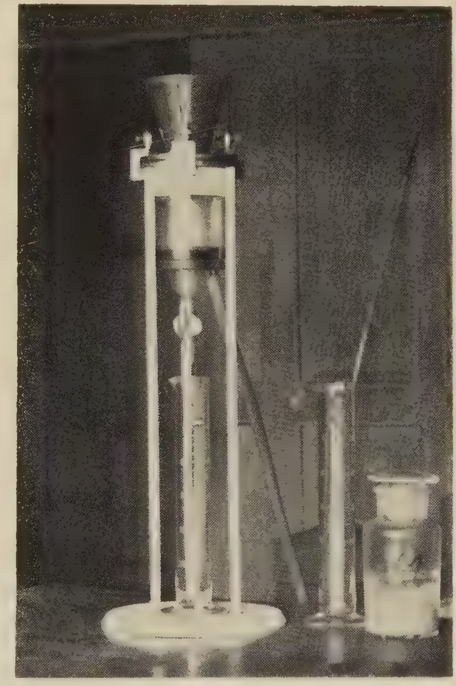


Fig. 16. Assemblage of plankton sample aspiration filtering apparatus (model 22).

The sample contained in a known volume of water (less than 200 cc) is poured into the upper funnel and the aspiration is set going. Organisms contained in the sample water are retained on the filter and the water passing the filter drops into the receptor. After switching off the aspirator, water concentrated in the receptor is let down into the graduated cylinder by opening the stopcock of the receptor. The difference between the volume of the original sample water and the water in the graduated cylinder is the displacement volume of plankton sample. Using fine bolting cloth, XX 13, 0.1 mm mesh size, as a filter, samples of ordinary size, say 15 cc of displacement volume, can be measured within a few

minutes when they include mainly copepods. Even the samples containing phytoplankton, as much as 15 cc of displacement volume, are filtered within ten minutes. Remaining organisms on the filter are almost drained forming a thick circular block like a cake. Their weight represents the wet weight of the samples.

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PLATES I–VIII

PLATE I

Fig. 17. Sketch of twin net with semi-circular openings (model 1)

b. d. ...... bottom disc
m. i. l. .... metal insert lock
n. .... net
s. r. ...... supporting rod
t. d. ...... top disc
w. c. ...... wire cable

Fig. 18. Sketch of continuous vertical sampler (model 2)

c. n. ...... conical net
d. s. g. .... double silk gauze band
p. d. w. .... propeller driven by water current
r. d. p. .... reel driven by propeller
s. f. m. ... space for flow-meter
s. s. g. .... single silk gauze band
s. w. s. .... spool winding silk gauze band
w. c. ...... wire cable
w. i. ...... water intake
w. o. ...... water outlet
w. s. ...... window with sieve

Fig. 19. Sketch of twin net with bent lid (model 3)

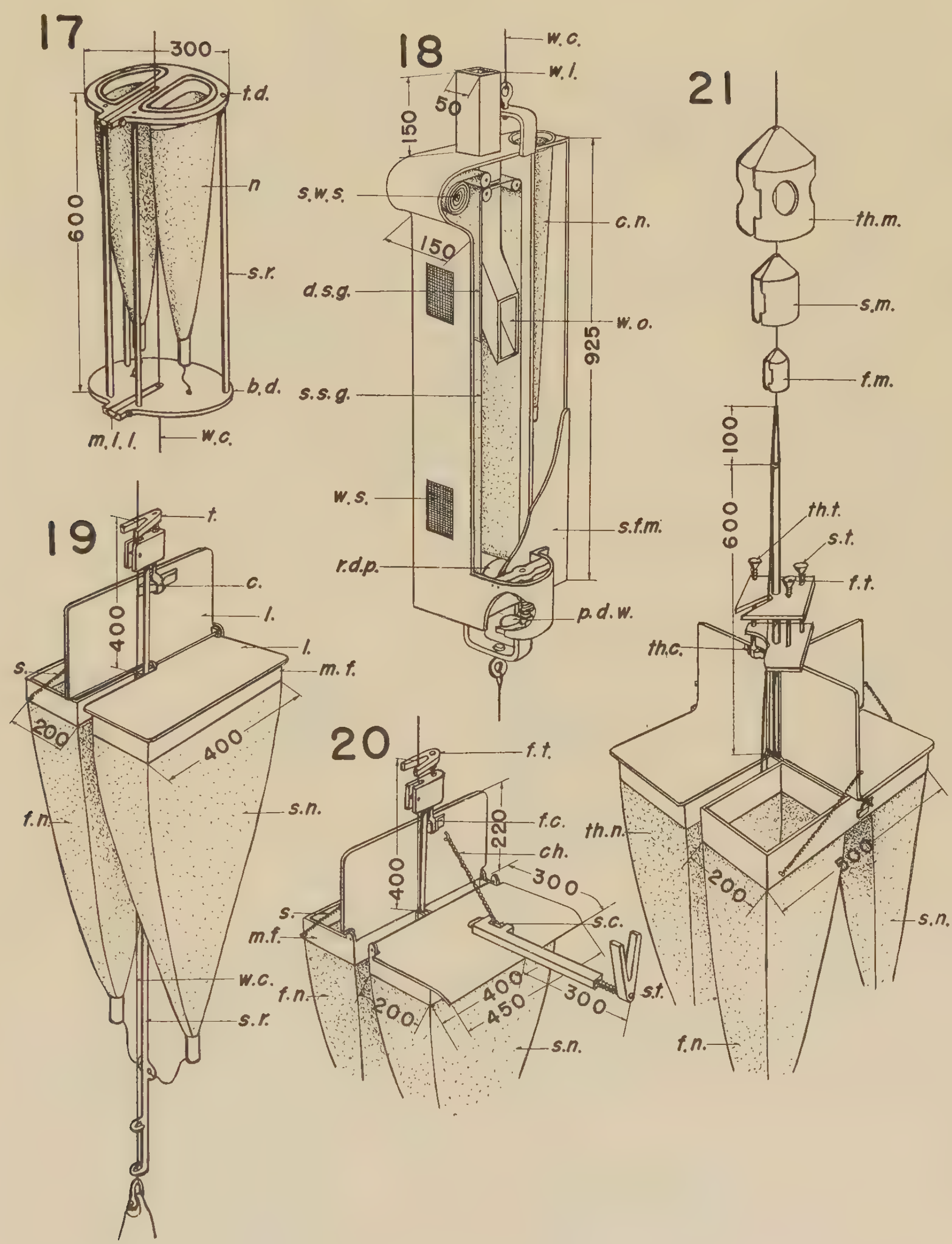
c. .... clamp
f. n. ...... first net
l. .... lid
m. f. ...... mouth frame
s. .... spring
s. n. ...... second net
s. r. ...... supporting rod
t. .... trigger
w. c. ...... wire cable

Fig. 20. Sketch of twin net for collecting in any two zones of water (model 4)

ch. ...... chain
f. c. ...... first clamp
f. n. ...... first net
f. t. ...... first trigger
m. f. ...... mouth frame
s. .... spring
s. c. ...... second clamp
s. n. ...... second net
s. t. ...... second trigger

Fig. 21. Sketch of four square net assembly (model 5)

f. m. ...... first messeenger
f. n. ...... first net
f. t. ...... first trigger
s. m. ..... second messenger
s. n. ...... second net
s. t. ...... second trigger
th. c. ..... third clamp. A part of square plate is removed to show the mechanism underside.
th. m. .... third messenger
th. n. ..... thrid net
th. t. ...... third trigger



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PLATE II

Fig. 22. Sketch of double purse net (model 6)

s. a. .... swinging arm

t. .... trigger

t. w. .... tripping weight

Fig. 23. Sketch of double closing net (model 7)

o. n. .... open net

r. m. .... releasing mechanism (Discovery type)

t. .... trigger

th. n. .... throttled net

Fig. 24. Sketch of horizontal tow with nets which are directly attached on the cable (model 8)

Fig. 25. Sketch of mouth part of horizontal net directly attached on the cable (model 8)

n. .... net

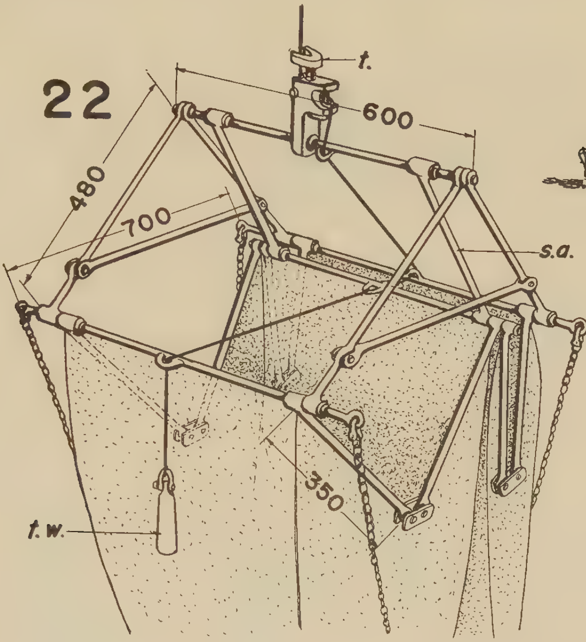
s. .... supporter

w. .... wire cable

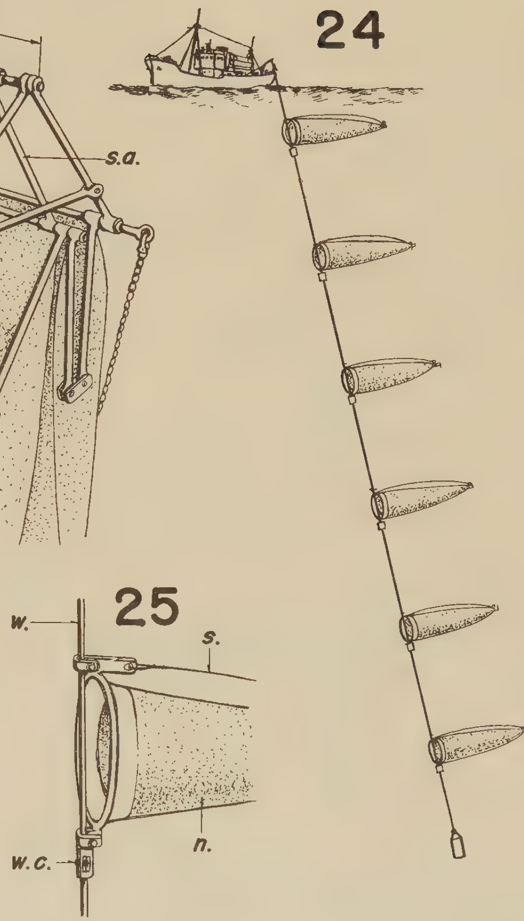
w. c. .... wire clamp

Fig. 26. Horizontal nets employed by Nishimura (After Nishimura 1957)

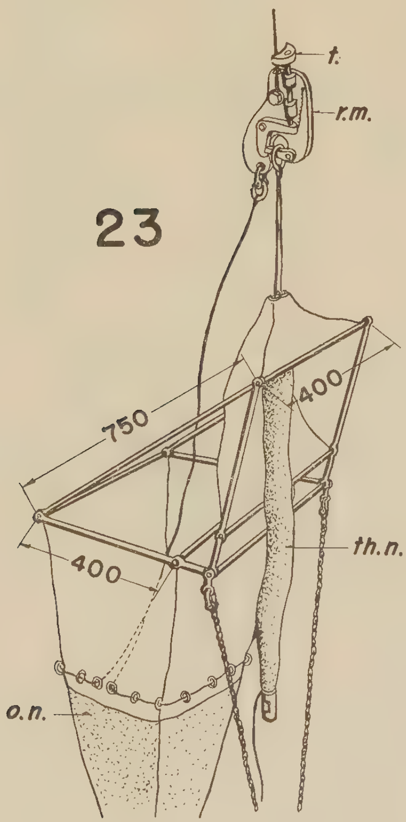
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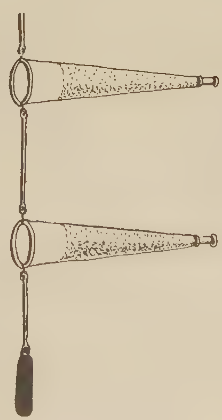


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PLATE III

Fig. 27. Sketch of horizontal net with sliding ring (model 9)

e. m. r. .... eye fixed on mouth ring
h. a. .... hinged arm
n. .... net
n. s. .... net supporter
s. r. .... supporting rod
w. .... wire cable

Fig. 28. Profile of frame of horizontal net with sliding ring (model 9)

e. .... eye fixed on mouth ring
h. a. .... hinged arm
m. r. .... mouth ring
s. r. .... supporting rod
w. .... wire cable

Fig. 29. Frontal view of frame of horizontal net with sliding ring (model 9)

e. .... eye fixed on mouth ring
h. a. .... hinged arm
m. r. .... mouth ring
s. r. .... supporting rod
w. .... wire cable

Fig. 30. Sketch of small horizontal net with rotary mouth ring (model 10) in lowering position

b. w. .... balance weight
m. r. .... mouth ring
n. .... net
r. .... rod
w. .... wire cable
w. c. .... wire clamp

Fig. 31. Sketch of small horizontal net with rotary mouth ring (model 10) in horizontal towing position

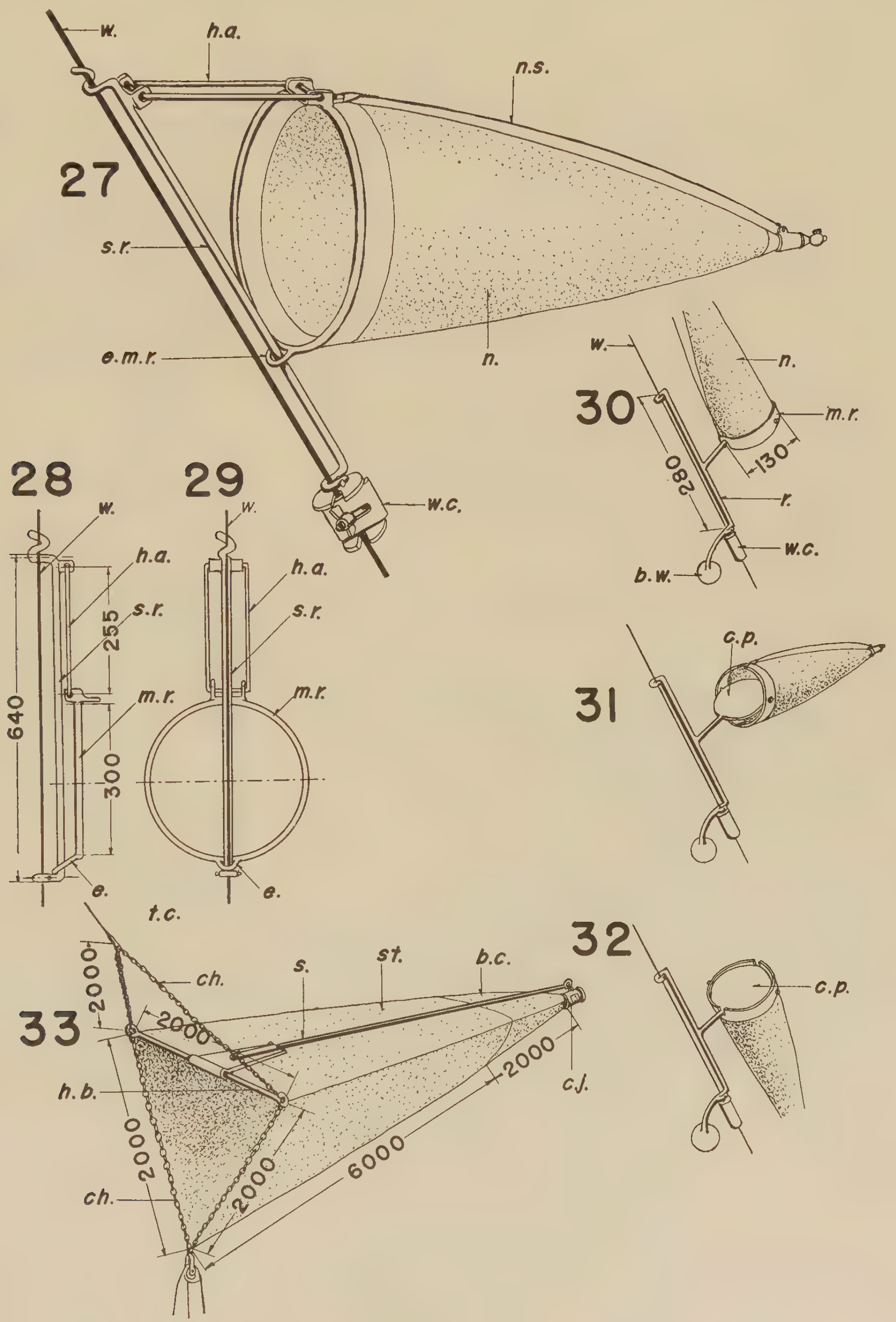
c. p. .... circular plate

Fig. 32. Sketch of small horizontal net with rotary mouth ring (model 10) in raising position

c. p. .... circular plate

Fig. 33. Sketch of triangular mid-water net (model 11)

b. c. .... bolting cloth
c. j. .... collecting jar
ch. .... chain
h. b. .... horizontal bar
s. .... supporter
st. .... stramin
t. c. .... towing cable



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PLATE IV

Fig. 34. Side view of double releasing mechanism with dissolving substanace (model 12)

cy.	cylinder	*f. p.*	follower piston
f. cy.	frame on cylinder	*f. r. a.*	first releasing arm
f. f. p.	frame on follower piston	*s. r. a.*	second releasing arm

Fig. 35. Plan of double releasing mechanism with dissolving substance (model 12)

c. b.	candy ball	*f. p.*	follower piston
cy.	cylinder	*f. r. a.*	first releasing arm
f. cy.	frame on cylinder	*s. r. a.*	second releasing arm
f. f. p.	frame on follower piston		

Fig. 36. Section A-B in Fig. 35

f. cy.	frame on cylinder	*f. p.*	follower piston
f. f. p.	frame on follower piston		

Fig. 37. Section C-D in Fig. 35.

cy.	cylinder	*f. f. p.*	frame on follower piston
f. cy.	frame on cylinder	*f. p.*	follower piston

Fig. 38. Section E-F in Fig. 35

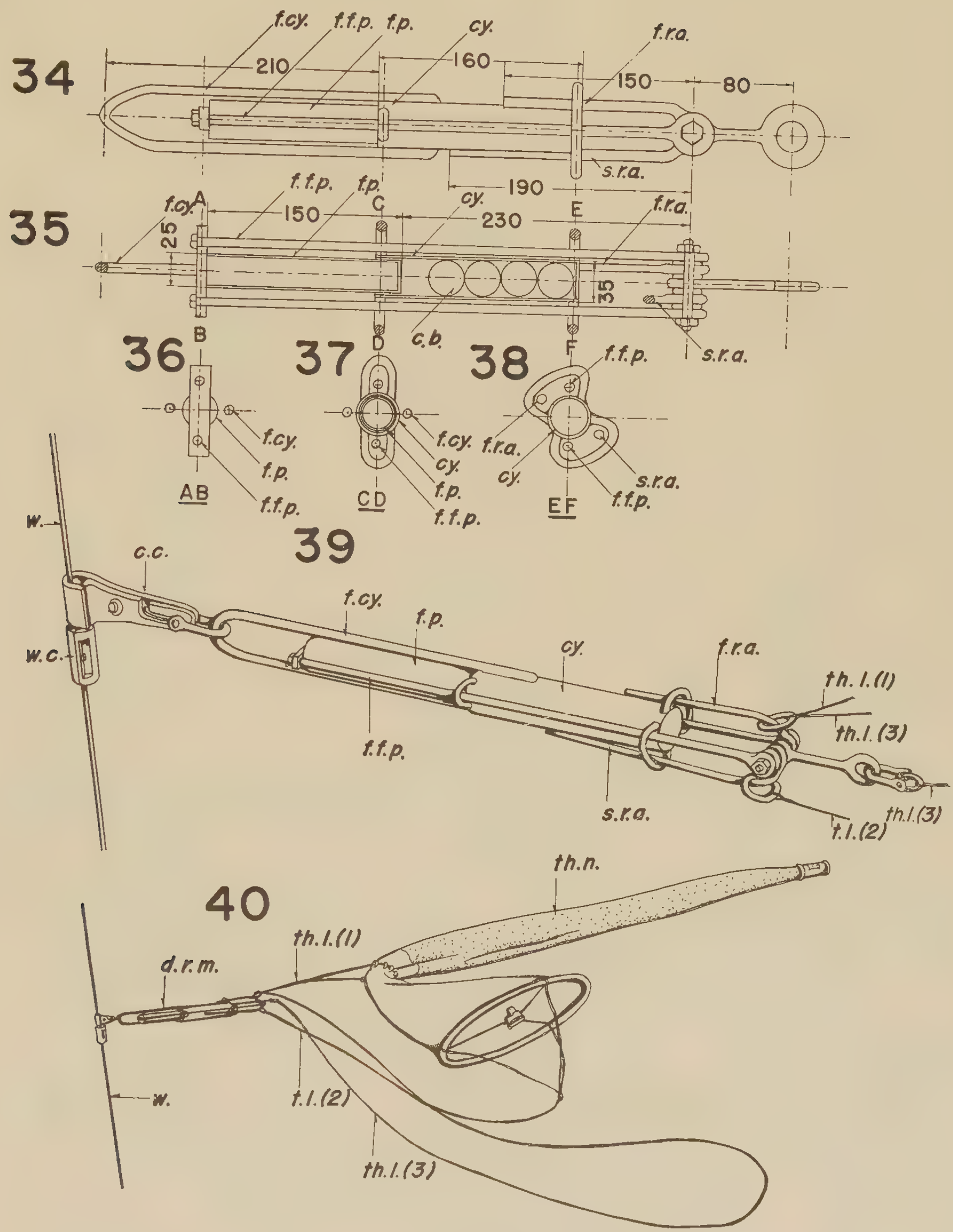
cy.	cylinder	*f. r. a.*	first releasing arm
f. f. p.	frame on follower piston	*s. r. a.*	second releasing arm

Fig. 39. Sketch of double releasing mechanism with dissolving substance (model 12) and cable connector

c. c.	cable connector	*s. r. a.*	second releasing arm
cy.	cylinder	*t. l.*	towing line
f. cy.	frame on cylinder	*th. l.*	throttling line
f. f. p.	frame on follower piston	*w.*	wire cable
f. p.	follower piston	*w. c.*	wire clamp
f. r. a.	first releasing arm		

Fig. 40. Sketch of opening-reclosing net set on the cable by means of double releasing mechanism with dissolving substance (model 12)

d. r. m.	double releasing mechanism	*th. l.*	throttling line
		th. n.	throttled net
t. l.	towing line	*w.*	wire cable



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PLATE V

Fig. 41. Plan of double releasing mechanism with long rotary neck (model 13)

f. c. .... first clamp
f. t. .... first trigger
s. c. .... second clamp
s. t. .... second trigger

Fig. 42. Sketch of double releasing mechanism with long rotary neck (model 13)

f. c. .... first clamp
f. t. .... first trigger
s. c. .... second clamp
s. t. .... second trigger
t. l. .... towing line
th. l. .... throttling line
w. .... wire cable
w. c. .... wire clamp

Fig. 43. Plan of double releasing mechanism with horizontal bar (model 14)

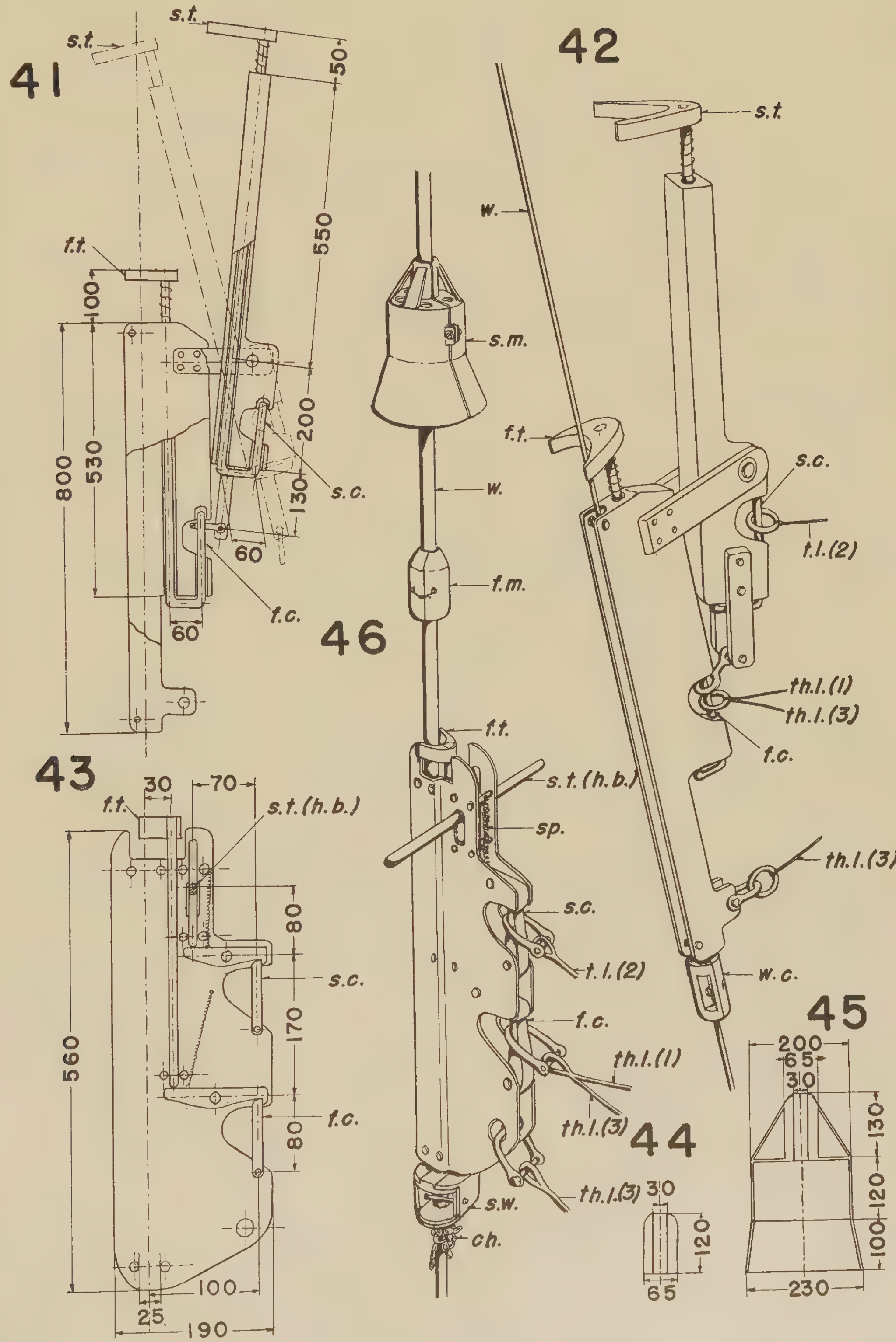
f. c. .... first calmp
f. t. .... first trigger
s. c. .... second clamp
s. t. (h. b.). .... second trigger (horizontal bar)

Fig. 44. Section of first messenger of double releasing mechanism with horizontal bar (model 14)

Fig. 45. Section of second messenger of double releasing mechanism with horizontal bar (model 14)

Fig. 46. Sketch of double releasing mechanism with horizontal bar (model 14)

ch. .... chain
f. c. .... first clamp
f. m. .... first messenger
f. t. .... first trigger
s. c. .... second clamp
s. t. (h. b.) .... second trigger (horizontal bar)
s. m. .... second messenger
s. w. .... spin washer
sp. .... spring
t. l. .... towing line
th. l. .... throttling line
w. .... wire cable



Motoda : Devices of Plankton Apparatus

PLATE VI

Fig. 47. Plan of high-speed tow net with heavy head (model 15)

cy. .... cylinder
h. r. ...... head rod
f. n. ...... folded net

Fig. 48. Frontal view of high-speed tow net with heavy head (model 15)

Fig. 49. Sketch of high-speed tow net with heavy head (model 15)

cy. .... cylinder
h. r. ...... head rod
f. n. ...... folded net
t. c. ...... towing cable

Fig. 50. Plan of simple underway plankton catcher IV (model 16)

h. p. ...... head piece
s. f. m. .... space for flow-meter
i. cy. ..... inner cylinder
s. p. ...... stopping pin
n. .... net
w. p. ..... water path
o. cy. ..... outer cylinder

Fig. 51. Frontal view of simple underway plankton catcher IV (model 16)

Fig. 52. Sketch of simple underway plankton catcher IV (model 16) showing connection of towing cable to the sampler in surface tow

t. c. ...... towing cable

Fig. 53. Sketch of simple underway plankton catcher IV (model 16) showing connection of line suspending depressor from the sampler in subsurface tow

d. .... to depressor
t. c. ...... towing cable

Fig. 54. Sketch of simple underway plankton catcher IV (model 16) showing attachment of the sampler in simultaneous tow with several samplers at different depths

t. c. ...... towing cable
w. c. ...... wire clamp

Fig. 55. Side view of simple underway plankton catcher VI with outside frame (model 17)

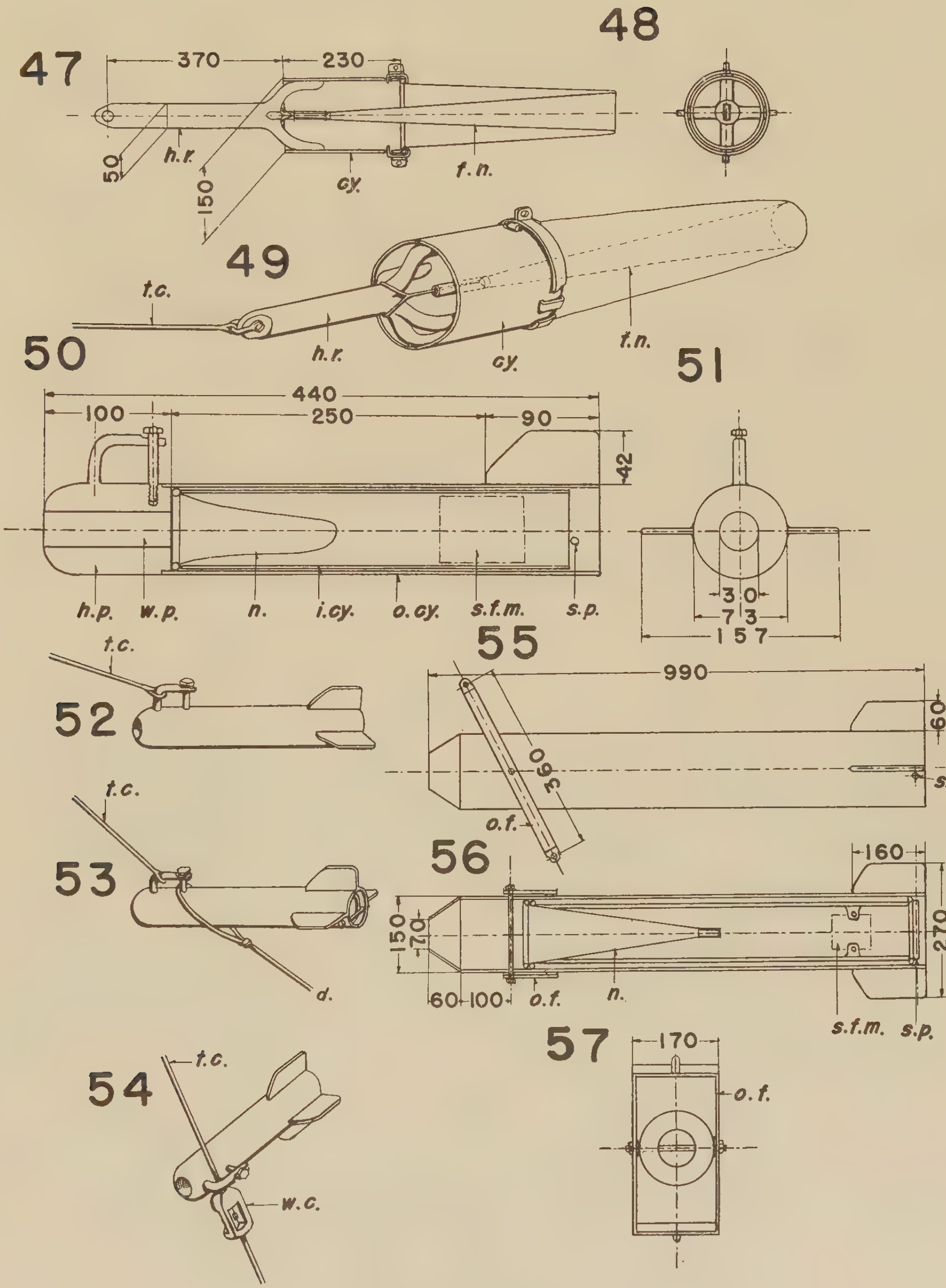
o. f. ...... outside frame
s. p. ...... stopping pin

Fig. 56. Plan of simple underway plankton catcher VI with outside frame (model 17)

o. f. ...... outside frame
s. f. m. .... space for flow-meter
n. .... net
s. p. ...... stopping pin

Fig. 57. Frontal view of simple underway plankton catcher VI with outside frame (model 17)

o. f. ...... outside frame



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PLATE VII

Fig. 58. Plan of multiple net underway sampler (model 18) showing path of water tunnel

f. r. ...... fore-reel	*w. t.* ...... water tunnel
r. r. ...... rear-reel	*w. i. c.* .... water intake cap

Fig. 59. Side view of multiple net underway sampler (model 18)

f. r. ...... fore-reel	*w. i. c.* .... water intake cap
n. .... net	*w. t.* ...... water tunnel
r. r. ...... rear-reel	

Fig. 60. Sketch of the anterior portion of multiple net underway sampler (model 18)

a. l. ...... accessory line	*sh. f. r.* ... shaft of fore-reel
a. o. ...... anterior opening	*t. c.* ...... towing cable
f. m. b. .... flexible metal band	*w. i. c.* .... water intake cap
n. .... net	*w. t.* ...... water tunnel

Fig. 61. Vertical of multiple net underway sampler with storing tank (model 19)

f. m. b. .... flexible metal band	*n. t.* ...... net turned inside out
f. r. ...... fore-reel	*r. r.* ...... rear-reel
f. t. ...... formalin tank	*sp.* .... spring
n. .... net	*w. t.* ...... water tunnel

Fig. 62. Plan of multiple net underway sampler with storing tank (model 19)

f. m. b. .... flexible metal band	*w. o.* ...... water outlet
f. r. ...... fore-reel	*w. t.* ...... water tunnel
r. r. ...... rear-reel	

Fig. 63. Section A-B in Fig. 61

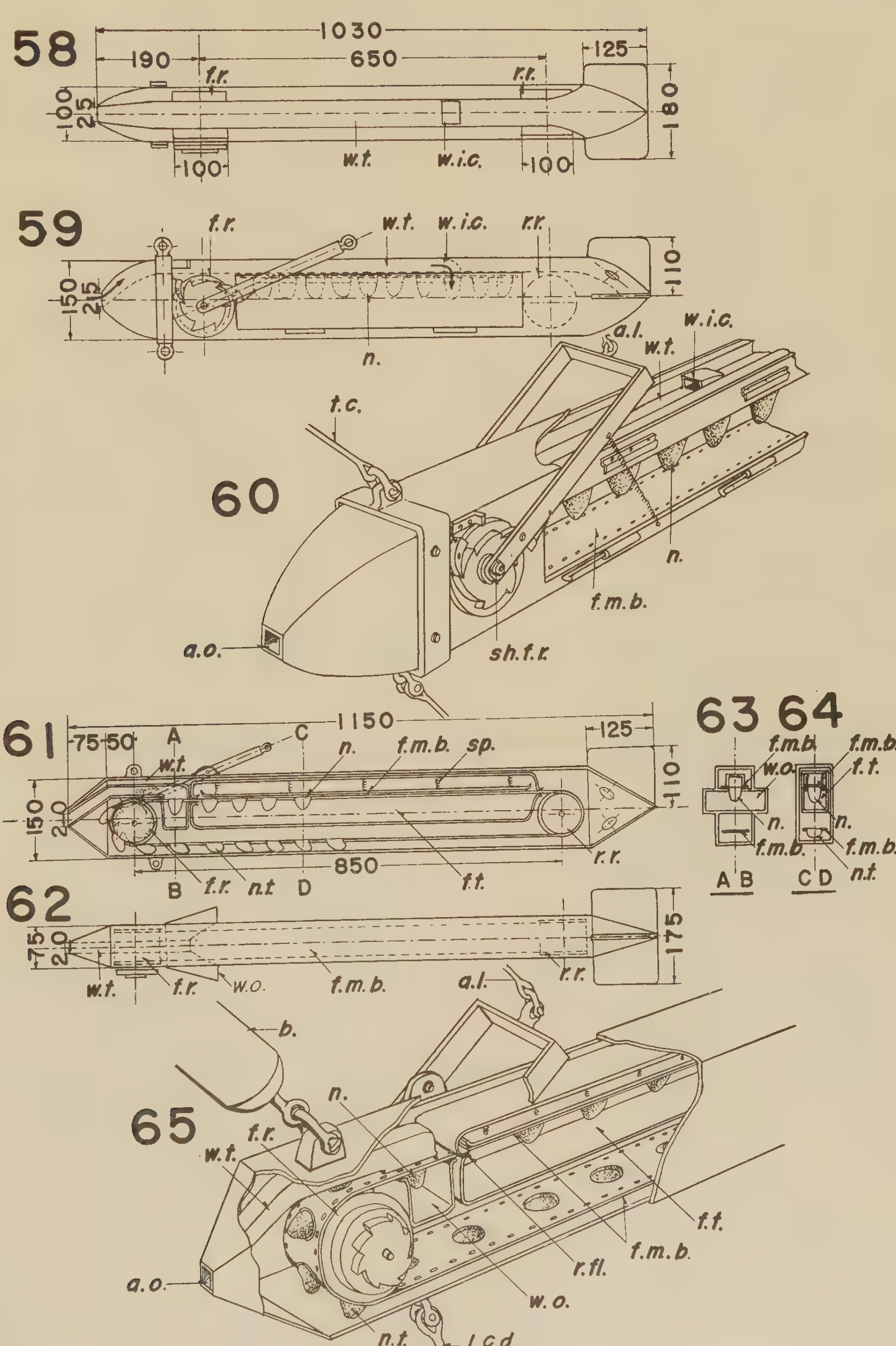
f. m. b. .... flexible metal band	*w. o.* ...... water outlet
n. .... net	

Fig. 64. Section C-D in Fig. 61

f. m. b. .... flexible metal band	*n.* .... net
f. t. ...... formalin tank	*n. t.* ...... net turned inside out

Fig. 65. Sketch of anterior portion of multiple net underway sampler with storing tank (model 19)

a. l. ...... accessory line	*n.* .... net.
a. o. ...... anterior opening	*n. t.* ...... net turned inside out
b. .... bumper	*r. fl.* ...... rubber flaps
f. m. b. .... flexible metal band	*w. o.* ...... water outlet
f. r. ...... fore-reel	*w. t.* ...... water tunnel
f. t. ...... formalin tank	
l. c. d. .... line connected to depressor	



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PLATE VIII

Fig. 66. Vertical of plankton sample splitting box (model 20)

b. p.	base piece	*l. r.*	low ridge
c.	cover	*s.*	septum

Fig. 67. Plan of plankton sample splitting box (model 20)

c.	cover	*l. r.*	low ridge
c. o.	corner opening	*s.*	septum

Fig. 68. Sketch of plankton sample splitting box (model 20)

b. p.	base piece	*l. r.*	low ridge
c.	cover	*s.*	septum
c. o.	corner opening		

Fig. 69. Vertical of plankton sample splitting cylinder (model 21)

b. p.	base plate	*s.*	septum
c.	cover		

Fig. 70. Plan of plankton sample splitting cylinder (model 21)

b. p.	base plate	*s.*	septum
c.	cover		

Fig. 71. Sketch of plankton sample splitting cylinder (model 21)

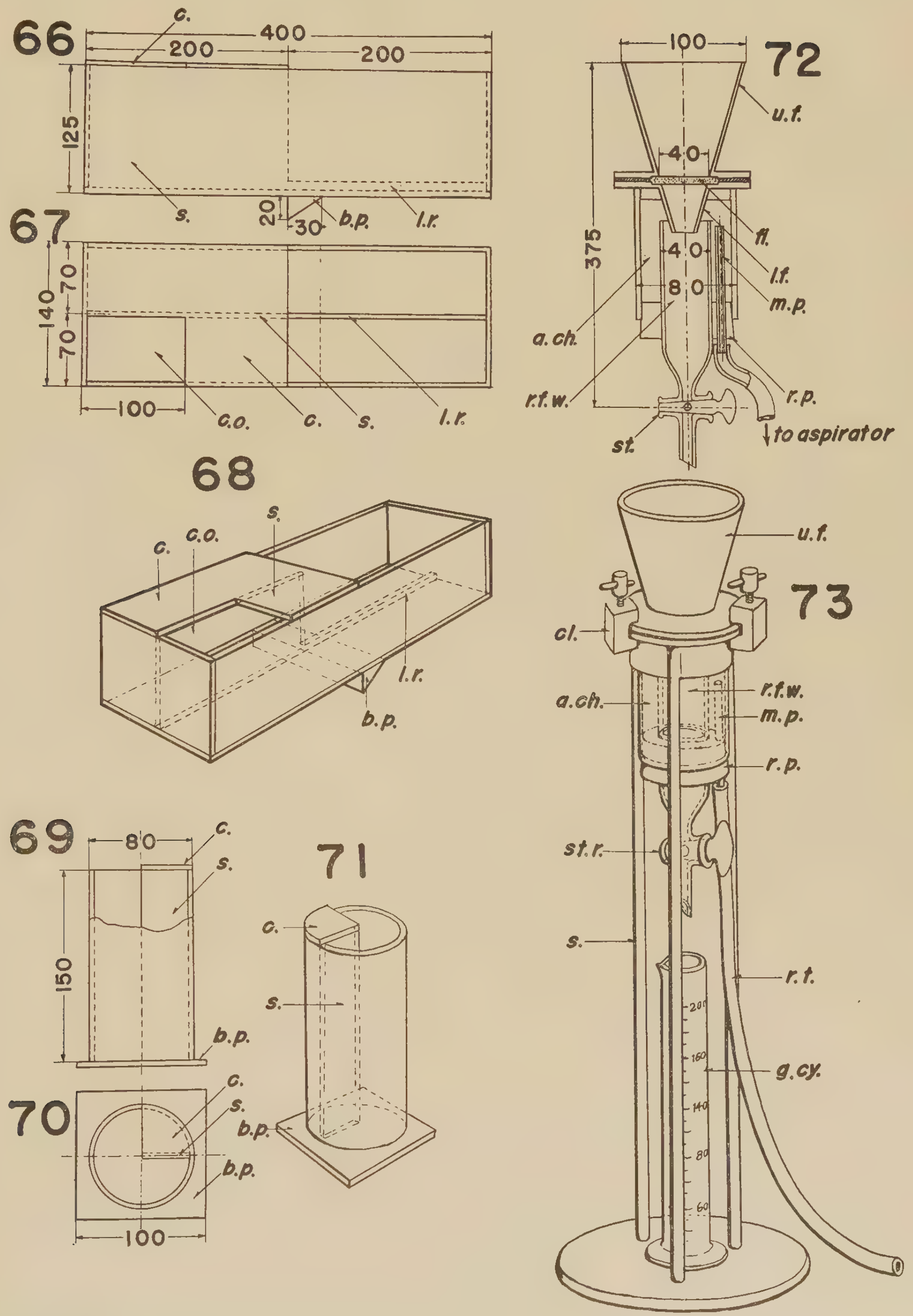
b. p.	base plate	*s.*	septum
c.	cover		

Fig. 72. Vertical of plankton sample aspiration filtering apparatus (model 22)

a. ch.	aspiration chamber	*r. f. w.*	receptor of filtered water
fi.	filter	*r. p.*	rubber plug
l. f.	lower funnel	*st.*	stopcock
m. p.	metal pipe	*u. f.*	upper funnel

Fig. 73. Sketch of assembly of plankton sample aspiration filtering apparatus (model 22)

a. ch.	aspiration chamber	*r. p.*	rubber plug
cl.	clip	*r. t.*	rubber tube connected to aspirator
g. cy.	graduated cylinder	*s.*	supporter
m. p.	metal pipe	*st. r.*	stopcock of receptor
r. f. w.	receptor of filtered water	*u. f.*	upper funnel



Motoda : Devices of Plankton Apparatus

STUDIES ON TECHNICAL PROBLEMS IN THE PROCESSING OF CANNED CRAB (*PARALITHODES CAMTSCHATICA* TILESIUS)

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CONTENTS

INTRODUCTION

1. Kinds of raw material of the canned crab

Economically important crabs produced in Japan are Alaska king crab (*Paralithodes camtschatica*), and "Ke-gani" (Horse-hair crab, *Erimacrus isenbeckii*). Besides, "Abura-gani" (*P. platypus*,) "Hanasaki-gani" (*P. brevipes*) and "Zuai-gani" (*Chinonectes opilio* (*O. fabricius*)) which are taken in less quantity, are processed for canning.

Alaska king crab is packed by Japanese packers on floating canneries or in land canneries. Other crabs are packed only in the land canneries.

The procedures in all cases are almost the same.

2. Processing of the canned crab

From the bodies of crabs which were captured by tangle nets (bottom gill nets) or by crab pots (for Horse-hair crab) the carapaces are removed.

Thus the legs are separated from the carapace. The carcasses from which the carapace were removed are put into netting bag made of manila twine with about 1.5 inch mesh; they are heated in boiling sea water in an iron tank. The boiling time is about 18～20 minutes for big crab (hard shell crab) when 200 carcasses are put in at one time, and about 17～18 minutes for small carcasses which can be put in at a rate of over 300 carcasses at one time.

After the cooked carcasses are removed from the cooking tank, they are cooled in cold sea water for about 10 minutes before further handling. The separating of the meat from the shell is done by a knife or scissors.

The meat separated from the shell is divided into body meat (shoulder meat), first leg meat (round meat), joint meat (so called scallion-type meat), second leg meat (so called pepper-type meat), claw meat, palm meat (scissor meat). The nomenclature of the body meat of *Paralithodes camtschatica* is shown in Fig. 1. Each type of meat is put into its own separate pan.

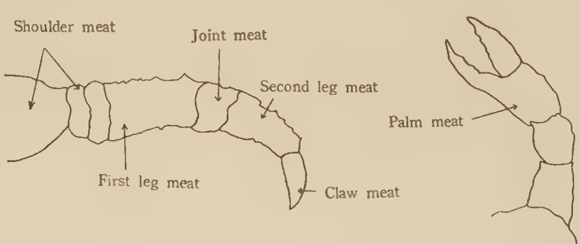


Fig. 1. Nomenclature of each type of body meat of *Paralithodes camtschatica*

After the separation of meat from the shell, the separated meat is transferred into the washing cages and washed in sea water in a tank.

After the washing, the shoulder meat holds its shape. The shoulder meat and leg meat which have gotten out of shape are degraded to "Flake meat".

The first and second leg meats are cut into segments of which the length is within the diameter of the can. The first leg meat holding its shape is used for the ornamental meat of the "First grade" (Fancy-style) canned crab. The second leg meat holding its shape is used for the ornamental meat of the "Third grade"-style canned crab.

The first and the second leg meats which have gotten out of shape are used as "Flake meat". The joint meat, and merus or wrist meats of cheliped are used for the "Flake meat" after deforming the shape. Palm meat (scissor meat) of cheliped is removed from the crust by crushing it.

Prepared crab meats are usually packed in parchment paper lined C-enameled 1/2-pound flat tin cans, of which the net content is 230 *g*. Occasionally a packer will use 1/4- or 1-pound flat can for special market.

The grades of canned crab are "Fancy", "Fair", "Third grade" ("Passed" A and B). In preparation of the "Fancy"-style cans, 2～4 large pieces of the first leg meat are placed in the top and bottom layers in order to show a good appearance; the shaped shoulder meat, and palm meat are placed in the sides. "Flake meat" is placed in the center. The "Fair" grade is the cans which were made as "Fancy-style", but the quality of the contents has not been passed as "Fancy" grade. The "Third grade" style is the cans in which the second leg meat or the broken meat (not flake meat) is placed in the top and bottom layers of the content, and "Flake meat" is placed in the center. When sea water is used for washing and boiling, the addition of salt is not needful. But when fresh water is used, salt must be added to the amount of about one *g* per 1/2-pound can. After the meat is packed in the can, the tops are clinched and then vacuum-sealed.

The sealed cans are placed in a retort where processing takes place for 80 minutes at a temperature of 227°F (108°C)～228.5°F (109°C) (5～5.5 pound pressure). After the processing, as soon as they are removed from the retort, cans must be cooled quickly.

The procedure for the canned horse-hair crab is almost the same as that for the canned king crab.

3. Scientific problems in the processing of canned crab

The crab industry is one of the important canning operations in Japan on the basis of export dollar value, so many studies have been undertaken from many years ago. By those studies, many technical problems or troubles have been solved. All the troubles which have occurred in the past and methods of preventing them are explained in a book by the present author, Eiichi TANIKAWA: "The processing of canned foods, (in Japanese), Kigensha, Tokyo".

The author has studied further the troubles and technical problems which have been called to his attention since the publication of that book.

For example, the author has reported in detail his studies on several problems in the

course of manufacture of canned crab from horse-hair crab (*Erimacrus isenbeckii*[1]). Recently, Kosakabe [2] (1957) has succeeded in preventing the appearance of the blue meat of the canned crab by "low temperature and fractional heating" of the carcasses from which carapace had been removed. In Japan the introduction of Kosakabe's method marks an epoch in the procedure of canning crab meat. However, resulting from the use of Kosakabe's method, derivative problems have occurred.

It is the purpose of this paper to describe the results of studies on those problems in the case of *Paralithodes camtschatica*.

Before going further the aurthor wishes to acknowledge with thanks the assistance rendered in this invesitgation through a grant in aid for the development of scientific research from the Minsitry of Education of Japan.

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I. Characteristics of raw king crab meat

1. *Velocity of bacterial decomposition of king crab meat*

It is well known that the crab meat is decomposable. The author[3] has previously reported the velocity of bacterial decomposition of the meat of *Paralithodes camtschatica* by estimating the amount of volatile basic nitrogen (V.B.-N) produced, and compared it with decomposition velocity of other marine creatures. According to the results obtained, the V.B.-N producing velocity constant (K), temperature constant (A) and temperature coefficient (Q_{10}) of the meat of those marine creatures are shown as in Table 1.

Table 1. V.B.-N producing velocity constant (K), temperature constant (A) and temperature coefficient (Q_{10}) of marine creatures

	King crab	Horse-hair crab	Salmon	Atka-mackerel	Mackerel	Saury	Squid	Sea-cucumber
$K\times10^3$ (30°∼37°C)	78	91	63	60	114	49	150	29
$K\times10^3$ (25°∼27°C)	—	74	38	36	99	30	103	28
Q_{10}	1.6	1.2	2.0	3.5	3.6	1.3	1.6	1.5
A	7,700	2,900	12,000	21,000	22,000	5,000	9,000	7,000

The values of $K\times10^3$ of king crab and horse-hair crab are larger than those of fish meat except mackerel meat, but they are smaller than the same value of squid meat.

Comparing king crab meat with horse-hair crab, the former shows less value of $K\times10^3$ than the latter, that is to say, the former is less decomposable than the latter. The values of "Q_{10}" and "A" of the fomer are larger than those of the latter. According to the results

previously obtained, when the amount of V.B.-N rises above 20 *mg*% in king crab meat, the meat becomes undesirable as does horse-hair crab meat also. The author has called this amount of V.B.-N that is 20 *mg*%, the limit of freshness of crab meat suitable for use as the raw material for canned food.

2. Why is the raw crab meat more decomposable than fish meat?

The author[4] has previously studied the chemical and physico-chemical properties of *Paralithodes camtschatica* meat.

According to the results obtained, it is remarkable that *Paralithodes camtschatica* meat has larger amount of water-content and less amount of crude fat than that of fish meat, but there is no difference of the protein-content between the two sorts of meat. The greater part of the total amount of nitrogen in *Paralithodes camtschatica* meat was extractive nitrogen in water soluble state. The amount of dissolved nitrogen substances are good media for the bacteria. One of the causes of the fact that the amount of dissolved nitrogen is larger may be the histological characteristics of the crab meat. From the histological observation, in the leg meat, the blocks of muscular fiber bundles are, respectively, surrounded by a thick layer of gelatinous tissue. This is different from the fish meat, in which the muscular fiber bundles are distributed closely and uniformly throughout the gelatinous tissue. Therefore, when the crab flesh is soaked in water, the blocks are deformed.

The soluble nitrogen substances are contained in net-work constructions in gelatinous tissue as body fluid. When bacteria invade into the crab meat, they may utilize the soluble nitrogen and multiply and deform the tissue.

The multiplied bacteria begin to play an imporant rôle in the decomposition of the crab meat.

II. Maximum storing time of PARALITHODES CAMTSCHATICA meat as raw material for canning

If unfresh raw material is empolyed, canned crab of good quality is not obtainable just as in other canned foods; then, what degree of the freshness of the raw material is the limit? And what is the limit number of hours as the maximum permissible storing time of *Paralithodes camtschatica* meat?

This storing time depends on the storing temperature. The author[1][3][5] has previously determined the amount of V. B.-N produced in raw meat of many kinds of fish meat, and has learned that when that amount of *Erimacrus isenbeckii* meat or *Paralithodes camtschatica* meat rises over about 20 mg%, the meat becomes unfit for canning.

The author[3], then, has studied the relation between storing temperature and maximum time ("t_{20}") during which *Paralithodes camtschatica* meat can be stored and still remain suitable as raw material for canning.

An equation has been devised showing the relation between the value of "log $t_{20\theta}$", the length of the time until which the amount of V.B.-N attained 20 *mg*% and the storing tem-

perature, θ.

$$\log t_\theta = \alpha - \beta\theta \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (1)$$

Here, "α" and "β" are constants.

This equation was applied for every sample which was treated by different operations, and the following equation was obtained for the raw meat with the crust.

$$\log t_\theta = 2.02 - 0.03\,\theta$$

From those data, the author has prepared a scale as shown in Fig. 2 in which showing the relation between storing temperature and maximum storing time of *Paralithodes camtschatica* meat as raw material for canned crab.

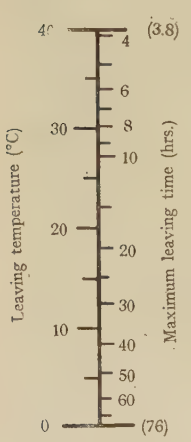


Fig. 2. Relation between storing temperature and maximum storing time of *Paralithodes camtschatica* meat

From Fig. 2, the maximum storing time (hrs.) for the raw fresh material can be learned. For example, at 15°C, the maximum storing time may be learned to be 26 hrs. It is advisable to know the maximum storing time for the raw fresh crab meat from the stand point of rational processing.

III. Maximum storing time of boiled crab meat for canning after heating at "high" or "low temperature"

The carcasses from which the carapace was removed are heated in sea water of high temperature or low temperature. Hitherto, the crab meat in the crust was boiled in boiling sea water, so workers could easily tear off the meat from the crust as a result of the hardening of meat protein.

Recently, in order to prevent the blueing of canned meat or to make canned boneless crab meat, the so called "low temperature boiling method" has been devised by Kosakabe[2]. By that "low temperature boiling method" (60°C for 20 minutes), myosin of the crab meat protein is coagulated, but haemocyanine, a blood protein, is not coagulated. The meat remains in half-boiled state. This meat can be pushed out by pressing of a roller or by water pressure. The pushed meat is soaked to allow flowing out of the blood containing haemocyanine. After having soaked for 10 minutes, the soaked meat is boiled for 3 minutes in sea water to harden it. Then the myogen in the meat protein coagulates in the same manner as that obtained by the usual boiling method. This boiling is called "Yudōshi" (hardening-boiling). The usual boiling method is called the "high temperature boiling method" in contrast to the "low temperature boiling method." When the crab meat boiled by the "high temperature boiling method" or "the low temperature boiling method" is left for a long time, the freshness of the meat becomes unfit for canning. The limit freshness of the crab boiled meat differs properly from that of the raw meat (20 *mg*% of V.B.-N). In fact, the degree

of the freshness of raw meat is scientifically not inspected, but that of the boiled meat is inspected by organoleptic test in canneries. It is rather necessary to know the limit of freshness of the boiled meat and the maximum storing time (hrs.) before the limit is reached.

The maximum storing time depends on the freshness of the meat before the boiling. The freshness of the boiled meat when treated by the "low temperature boiling method" falls depending on the storage temperature and the length of storage before the "hardening-boiling", because this boiled meat treated by "low temperature boiling method" is decomposable by autolytic enzyme. Study was made on the variation of the freshness during the storing after the crab meat was treated by the method of "high temperature" or "low temperature boiling". The limit of freshness of the boiled meat was determined.

1. Variation of freshness of the boiled crab meat treated by the "high temperature" or "low temperature boiling method"

(1) Experimental method

Crab which was captured off Saghalein was classified into two groups: hard shell crab and soft shell crab. The two groups of the material were divided into fresh and unfresh crab. The amount of V.B.-N in fresh hard shell crab meat was 6.3 *mg*%, while that in fresh soft shell crab meat was 6.2 *mg*%, and that in unfresh hard shell crab meats was 16.7, 25, 33.7 *mg*%, respectively (in this case soft shell crab was not employed). A group of samples of those meats having crust which was fresh or unfresh was boiled by the "high temperature boiling method" (100°C, 18 minutes). After the boiling, the meat was left at room temperature (5°∼10°C) or in an ice box (3°C), and was estimated for the amounts of V.B.-N and amino-N, pH and bacterial counts. The amount of V.B.-N was estimated by Conway's method, that of amino-N was by Pope-Stevens' method and pH value by a glass electrode apparatus. The bacterial count was done by decimal dilution method employing Burri's tube in order to develop anaerobic thermotolerant bacteria.

Another group of crab carcasses was heated by the "low temperature boiling method" (60°C, 10 minutes); this half-boiled meat was pushed out from the crust and soaked in cold water for 10 minutes; the soaked meat was boiled at 100°C for 3 minutes by "hardening-boiling". The meat was left at room temperature (5°∼10°C), or at 3°C and 0°C (in refrigerator).

(2) Experimental results

Results obtained are shown in Tables 2∼13 and Figs. 3∼6.

As seen in Tables 2∼13 and Figs. 3∼6, the amounts of V.B.-N and amino-N in fresh crab meats boiled by "low temperature boiling method" increased larger and more rapid than those by "high temperature boiling method".

This may be caused by autolytic action and bacterial action which were remaining even after subjection to "low temperature boiling method", and because of the smaller

Table 2. Variation in the freshness of fresh hard shell crab (V.B.-N, 6.3 mg%) subjected to "high temperature boiling method" and left at 3°∼5°C

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Amino-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.6	6.3	—	5×10^2	Good frerhness
(After boiling)					
2	6.7	10.9	68	2.5×10^2	Normal
6	6.7	15.8	113	2.5×10^2	〃
20	6.8	16.3	136	3.5×10^2	Meat was slightly softened
40	6.8	17.2	209	2.4×10^3	Meat was softened, Color was yellowish
52	6.9	20.2	200	3.5×10^4	Ibid., Off flavour
60	6.9	—	—	6.5×10^4	Same as above

Table 3. Variation in the freshness of fresh soft shell crab (V.B.-N, 6.2 *mg*%) subjected to "high temperature boiling method" and left at 3°∼5°C

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Amino-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.6	6.2	—	5×10^2	Good freshness
(After boiling)					
2	6.7	10.8	106	1×10^2	Normal
6	6.8	11.2	137	—	〃
20	6.9	12.0	144	3.3×10^2	〃
40	7.0	15.5	—	1.4×10^3	Meat was slightly softened
52	7.0	19.5	—	2.4×10^2	Off flavour
60	7.0	—	—	—	

Table 4. Variation in the freshness of pretty unfresh hard shell crab (V.B.-N, 16.7 *mg*%) subjected to "high temperature boiling method" and left at 0°∼5°C

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.2	16.7	2×10^3	Pretty fresh
(After boiling)				
2	6.4	12.0	1×10^3	Normal
24	6.4	15.0	2×10^3	Color was slightly yellowish
48	6.6	16.4	5×10^3	Meat was slightly softened
52	6.7	19.1	6×10^4	Ibid., Slightly stench
72	6.7	23.4	—	Stench
100	6.9	28.5	—	Ibid.

Table. 5. Variation in the freshness of unfresh hard shell crab (V.B.-N, 25 *mg*%) subjected to "high temperature boiling method" and left at 5°∼10°C

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.4	25.0	5×10^3	Pretty fresh
(After boiling)				
0	6.4	7.1	2×10^3	Meat was softened. Off flavour
3	6.2	5.4	2×10^3	Ibid. Color was yellowish
48	6.4	7.2	5×10^3	Same as above
56	6.6	12.3	2×10^5	Slightly putrefactive odour
72	7.6	80.5	above 10^6	Remarkably putrefactive odour
96	8.2	106.7	〃	Ibid.

Table 6. Variation in the freshness of very unfresh hard shell crab (V.B.-N, 33.7 *mg*%) subjected to "high temperature boiling method" and left at 5°~10°C

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.8	33.7	1×10^4	Unfresh
(After boiling) 0	6.8	9.3	8×10^3	Meat was softened. Color was yellowish. Slightly putrefactive odour
24	7.1	17.3	2×10^4	Same as above
48	7.2	22.2	3×10^4	〃
72	7.2	24.6	3×10^5	Remarkably putrefactive odour
96	7.4	25.8	10^6	Same as above

Table 7. Variation in the freshness of very unfresh hard shell crab (V.B.-N, 33.7 *mg*%) subjected to "high temperature boiling method" and left at 0°C

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.8	33.7	1×10^4	Unfresh
(After boiling) 0	6.8	9.3	8×10^3	Putrefactive odour. Meat was softened
24	7.2	13.4	1×10^4	Same as above
48	7.2	12.6	1×10^4	〃
72	7.4	12.3	1×10^4	〃
96	7.2	13.7	2×10^4	〃
120	7.2	12.3	2×10^4	〃
144	7.2	18.1	5×10^4	〃

Table 8. Variation in the freshness of pretty unfresh hard crab (V.B.-N, 16.7 *mg*%) subjected to "low temperature boiling method" and left at 0°~5°C for various lengths of time

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.2	16.7	2×10^3	Pretty unfresh
(After boiling) 2	6.2	10.4	1×10^3	Normal
24	6.4	10.8	3×10^3	〃
48	6.5	14.3	2.5×10^3	Meat color was yellowish
52	6.6	19.4	5×10^4	Meat was softened. Off odour
72	6.7	21.6	—	Same as above
100	6.8	27.9	—	Putrefactive odour

Table 9. Variation in the freshness of unfresh hard crab (V.B.-N, 25 *mg*%) subjected to "low temperature boiling method" and left at 5°~10°C for various lengths of time

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.4	25.0	5×10^3	Unfresh
(After boiling) 0	6.4	8.2	2×10^3	Meat was softened. Off odour
3	6.0	6.0	2×10^3	Ibid. Color was yellowish
48	6.4	8.2	8×10^3	〃
56	6.7	14.9	5×10^5	Same as above
72	7.2	73.7	above 10^6	Putrefaction
96	8.4	131.0	〃	〃

Table 10. Variation in the freshness of fresh hard crab (V.B.-N, 6.3 *mg*%) subjected to "low temperature boiling method" and left for various times at 3°~5°C before and after hardening boiling

By low-temp. boiling method at 60°C for 10 mins.						Hardening boiling at 100°C for 3 mins.	Leaving time after the hardening boilng								
							4 hrs.			20 hrs.			54 hrs.		
Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Amino-N (*mg*%)	Bacterial counts (per *g*)	Appearance		pH	V.B N (*mg*%)	Bacterial counts	pH	V.B.-N (*mg*%)	Bacteril counts	pH	V.B.-N (*mg*%)	Bacterial counts
Before boiling	6.6	6.3	—	5×10^2	Fresh										
(After boiling) 2	6.3	10.1	136	3.3×10^2	Normal										
6	6.7	16.4	189	5.0×10^2	〃	——→	6.5	10.5	1×10^2	6.6	10.8	2×10^2	6.7	14.0	1×10^4
20	6.8	17.7	212	8.6×10^3	Meat was softened	——→	6.6	11.0	1×10^2	6.6	11.0	1×10^3	6.8	15.9	3×10^4
40	6.8	22.0	240	4.5×10^3	〃	——→	6.6	11.0	1×10^3	6.6	11.2	—	6.8	18.8	5×10^4
60	7.2	50.8	—	3.5×10^5	Putrefactive odour										

Table. 11. Variation in the freshness of fresh soft shell crab (V.B.-N, 6.2 *mg*%) subjected to "low temperature boiling method" and left for various times at 3°~5°C before and after hardening boiling

By low-temp. boiling method at 60°C for 10 mins.						Hardening boiling at 100°C for 3 mins.	Leaving time after the hardening doiling								
							4 hrs.			20 hrs.			54 hrs.		
Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Amino-N (*mg*%)	Bacterial counts (per *g*)	Appearance		pH	V.B.-N (*mg*%)	Bacterial counts	pH	V.B.-N (mg%)	Bacterial counts	pH	V.B.-N (*mg*%)	Bacterial counts
Before boiling	6.6	6.2	—	5×10^2	Fresh										
(After boiling) 2	6.2	7.9	159	5.3×10^2	Normal										
6	6.5	10.4	170	6.3×10^2	〃	——→	6.4	7.4	5×10^2	6.5	10.3	2×10^3	6.6	11.9	3×10^3
20	6.6	13.5	190	3.5×10^3	Meat was softened. Off flavor	——→	—	—	—	6.6	12.3	3×10^3	6.7	16.5	1×10^4
40	6.9	22.4	257	4.5×10^3	Slightly putrefactive odour	——→	—	—	—	6.6	12.9	3×10^3	6.8	19.0	1×10^4
60	7.0	43.7	325	4.0×10^5	Putrefaction										

Table 12. Variation in the freshness of very unfresh hard crab (V.B.-N, 33.7 *mg*%) subjected to "low temperature boiling method" and left at 5°～10°C for various lengths of time

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.8	33.7	1×10^4	Unfresh
(After boiling)				
0	6.8	14.2	8×10^3	Meat was softened. Putrefactive odour
24	7.0	10.3	1×10^4	〃
48	7.2	21.1	2×10^4	〃
72	7.4	25.4	8×10^4	〃
96	33.6	33.6	1×10^6	〃

Table 13. Variation in the freshness of very unfresh hard crab (V.B.-N, 33.7 *mg*%) subjected to "low temperature boiling method" and left at 0°C for various lengths of time

Leaving time (hrs.)	pH	V.B.-N (*mg*%)	Bacterial counts (per *g*)	Organoleptic observation
Before boiling	6.8	33.7	1×10^4	Unfresh
(After boiling)				
0	6.8	14.2	1×10^4	Meat was softened, Off odour
24	7.2	14.7	1×10^4	Slightly putrefactive odour
48	7.4	17.8	3×10^4	Putrefactive odour
72	7.4	17.2	3×10^4	〃
96	7.7	23.5	8×10^4	〃
120	7.5	23.2	1×10^5	〃
144	7.6	24.6	1×10^5	〃

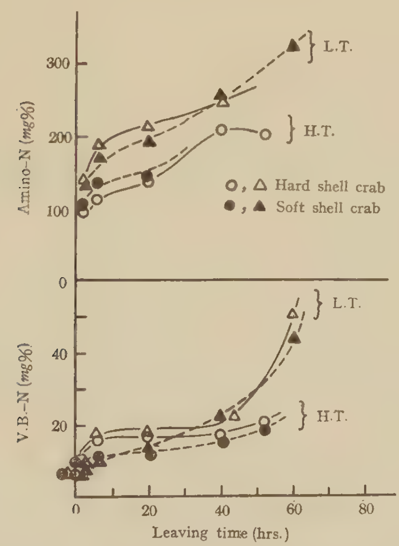


Fig. 3. Variation of the amount of V.B.-N and amino-N in the hard or soft shell crab meats subjected to "high" or "low temperature boiling method" respectively and left at 5°C

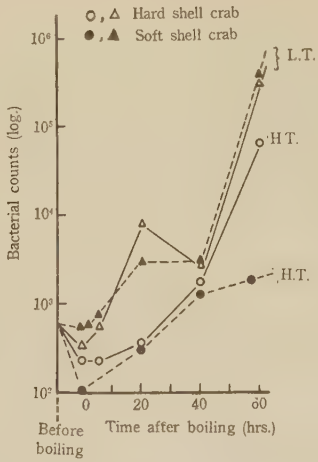


Fig. 4. Variation of the bacterial number in hard or soft shell crab meats subjected to "high" or "low temperature boiling method" respectively and left at 5°C

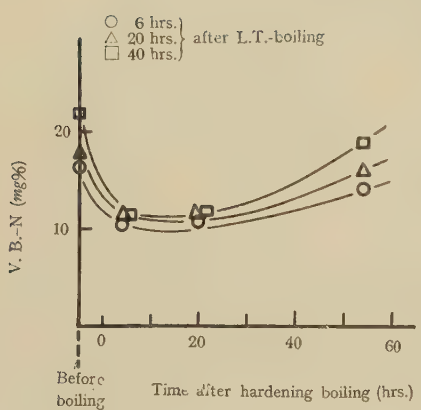


Fig. 5. Variation of the amount of V.B.-N in frsh hard crab meat (V.B.-N, 6.3 *mg*%) subjected to "low temperature boiling method" and then treated by "hardening boiling" after various tmies and left

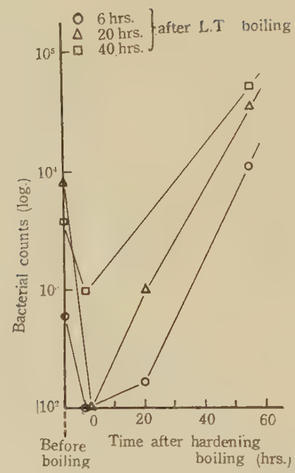


Fig. 6. Variation of the bacterial counts in fresh hard crab meat (V.B.-N, 6.3 *mg*%) subjected to "low temperature boiling method" and then treated by "hardening boiling" after various times and left

amount of dehydration; consequently the nutrient components for bacteria remain in the meat.

Therefore the boiled crab meat prepared by "low temperature boiling method" must be more rapidly transferred to the next procedure than that subject to "high temperature boiling method". It is remarkable that on V.B.-N producing curves of the boiled crab meat, the increaseing velocity of the produced amount of V.B.-N is gradual (lag period of the production curve is comparatively long) and it increases rapidly after the amount of V.B.-N reached to about 20 *mg*%.

But the amount of amino-N increases rapidly from the initial storing period. The amount of V.B.-N and the bacterial counts increase in boiled crab meat treated by "low temperature boiling method" after long storing time, but they decrease after the "hardening-boiling". However, the once decreased amount of V.B.-N and bacterial counts increase with prolongation of the storing time.

Even if the boiled meat prepared by "low temperature boiling method" which was left for a long time, shows less than 20 *mg*% of V.B.-N, the meat becomes soft and emits decomposed odour. If the boiled crab meat prepared by "low temperature boiling method" is left for a long time, the amount of V.B.-N and the bacterial counts after the "hardening boiling" are larger than that if left for a short time; the increase after the "hardening boiling" is larger in long storing than in short storing.

2. The limit freshness of boiled crab meats prepared by "high" or "low temperature boiling method", when the raw meat has various degree of freshness before the boiling

In the previously described experiment, the author has compared the decomposing velocities of the boiled crab meats which were treated by "high temperature boiling," and were left for various periods of time. The boiled crab meats having various degrees of freshness were processed for canning. The quality of the canned crab thus processed was inspected, and the limit of the freshness of the boiled crab meat was determined from the view point of suitability as material for canning.

(1) Experimental method

Raw crab meats having various degrees of freshness (the amounts of V.B.-N were 4.8 *mg*%, 17.0 *mg*%, and 36.9 *mg*%, respectively) as shown in Table 14, were subjected to the "high temperature boiling method" (at 100°C, 18 minutes) or to the "low temperature boiling method" (at 65°C, 10 minutes) and were left at 0°～3°C, 10°～13°C from 0 hour to 96 hours. Those boiled crab meat samples were divided into two groups. One of them was estimated for pH of the meat, amount of V.B-N and organoleptic test as definite intervals of storing time. The other group was processed for canning respectively at the same intervals. In the case of use of the "low temperature boiling method", after respective storing times, the boiled meats were subjected to "hardening boiling" (at 100°C, 3 minutes), and processed for canning as usual (sterilizing temperature was 6 pound-pressure for 95 minutes).

Table 14. Record of crab meat employed in the experiments

Degrees of freshness of raw meat (V.B.-N, *mg*%)	4.8, 17.0, and 36.9 *mg*%
Boiling method	High temp. boiling method and low temp. boiling method
Leaving temperature after boiling	0°～3°C and 10°～13°C
Leaving time after boiling	0, 24, 48, 72 and 96 hrs.

The cans processed thus were stored for a month in the laboratory and then opened. The quality of cans was examined for pH, amounts of V.B.-N, aminoN-, reducing sugar, and color of the meat; odour components test was also applied.

(2) Experimental results

According to the inspection of the quality of the canned crab processed as above described, the cans which were from the raw crab meat having below 20 *mg*% of V.B.N- and were treated by the "high temperature" or "low temperature boiling method", and were then processed, all had good quality. But those cans which were processed from the raw material having above 20 *mg*% V.B.-N and subjected to exactly the same method as

above described, were ascertained to be not good in quality: in texture, taste, odour, nor in color of the content meat. Those results were agreed with the results obtained previously by the author[3]. Even if the raw fresh crab meat was employed, with the lengthening of storage time after boiling, the quality of the contens of the cans becomes worse. In this case, the canned crab meat which was processed from the boiled meat treated by "low temperature boiling method" falls in quality more rapidly than that processed by "high temperature boiling method". With rising of the storing temperature afte the boiling, the quality falls more rapidly than in the case of the comparatively lower storing temperature.

As to the odorous components in stored boiled crab meat, the author has found cadaverine, lactic acid, propionic acid, iso-butylaldehyde, formaldehyde, acetaldehyde besides the components found in normal canned crab (methylamine, trace of formic acid and acetic acid).

From the results obtained in the present experiment, the limit of freshness of the boiled crab meat for good quality cans may be shown in terms of the amount of V.B.-N as recorded in Table 15. That is to say, when the freshness of the raw crab meat is below 10 *mg*% V.B.-N, the limit of the freshness of the boiled meat is 15 *mg*%; and when the

Table 15. Relation between the degrees of freshness of raw crab meat and the limit of freshness of boiled crab suitable for the material of canning.

Degrees of freshness of raw material (V.B.-N, *mg*%)	Limit of freshness of the boiled material
Below 10 *mg* %	Below 15 *mg* %
10~20 *mg* %	10~13 *mg* %

freshness of the raw crab meat is 10~20 *mg*%, the limit of the freshness of the boiled meat should be 10~13 mg%.

When the freshness of the raw crab meat is above 20 *mg*%, even if the raw meat is boiled and processed for canning without delay, good quality of canned crab is not obtainable. This accords with previous results.

3. Relation between storing temperature of the boild crab meat and maximum storing time

The maximum storing time before the limit of the freshness of the boiled crab meat is reached, depends on the storing temperature. It is also advisable to know the maximum storing time for the boiled crab meat at each storing temperature from the standpoint of rational processing.

Under this heating, the author will discuss the relation between the maximum storing time before the limit of the freshness of the boiled crab meat is reached and the storing temperature of basis on the experimental results reported in the previous articles: eight

sets of results are shown in Table 16.

Table 16. Experimental program for study on the relation between storing temperature of the boiled crab meat and maximum storing time

Experiments	Freshness of raw material (V. B.-N, *mg*%)	Boiling method	Leaving temp. after boiling
A	6.3	(1) H. T. (2) L. T.	3°C
B	6.3	(1) H. T. (2) L. T.	13°C
C	16.7	(1) H. T. (2) L. T.	3°C
D	17.1	(1) H. T. (2) L. T.	13°C

From the variations in the amount of increase of V.B.-N in the boiled crab meats which are shown in Table 16, the velocity constant of the V.B.-N production curve were calculated from equation (2) of monomolecular reaction after Kimata[6].

$$\log y/A - y = Kt + C \quad \cdots\cdots\cdots\cdots (2)$$

Here, "y" is the increased amount of V.B.-N after "t" hours. "A" is the maximum amount of V.B.-N produced. "C" is a constant, and "K" is the velocity coefficient. Then, temperature coefficient "Q"$_{10}$ is represented by equation (3).

$$\frac{K_{\theta+10}}{K_\theta} = Q_{10} \quad \cdots\cdots\cdots\cdots\cdots\cdots (3)$$

Here, "K_θ" and "$K_{\theta+10}$" are velocity constants at the temperatures θ°C and $(\theta+10)$°C, respectively. From equation (3), the temperature coefficient "Q_{10}" was calculated. The results calculated are shown in Table 17

Table 17. Relation between the values of "K" and "Q_{10}" when boiled crab meats treated by both "high" and "low temperature boiling methods" were stored at various temperatures

Freshness of raw material (V. B.-N, *mg*%)	Boiling method	Leaving temp.	$K\times10^3$	Q_{10}
6.3	H. T.	3°C 13°C	4 10	2.5
6.3	L. T.	3°C 13°C	13 30	2.3
16.9	H. T.	3°C 13°C	26 60	2.3
16.9	L. T.	3°C 13°C	29 63	2.2

As seen in Table 17, the V.B.-N production velocity constant, "K", of the boiled crab meat is larger at higher storing temperature than at lower storing temperature. It is also larger in the boiled crab meat prepared by "low temperature boiling method" than that prepared by "high temperature boiling method". It is also larger in the boiled crab meat which was unfresh raw material than in that which was fresh.

That is to say, it was learned that the boiled crab prepared meat by "low temperature boiling method" is more decomposable than that prepared by "high temperature boiling method", and that the boiled meat made from unfresh raw crab meat is more decomposable than that made from fresh.

There was no difference between the values of the temperature coefficient, "Q_{10}", of the boiled crab meat subjected to "high temperature boiling method" and that by "low temperature boiling method". The mean value of "Q_{10}" was 2.3. Considering on the basis of the inspection standard previously proposed, that the limit freshness of the boiled crab meat is about 15 *mg*% of V.B.-N, when the raw crab was below 10 *mg*% of V.B.-N, and about 12 *mg*% when 10～20 *mg*%, the time required to reach the limit freshness, "t_{15}" or "t_{12}" can be obtained for the boiled crab meats treated by "high" or "low" boiling methods which have had various degrees of freshness of raw meat and which were left at 3°C.

The results obtained are shown in Table 18.

Table 18. The values of "t_{15}" and "t_{12}" of boiled crab meats which were subjected to "high" and "low temperature boiling methods" and stored at 3°C

Freshness of raw material (V. B. -N *mg* %)	Boiling method	t_{15} of t_{12}
6.3	H. T.	$t_{15} = 6.5$
	L. T.	$t_{15} = 5$
16.9	H. T.	$t_{12} = 4$
	L. T.	$t_{12} = 3.5$

From Table 18, the value of "t_{15}" or "t_{12}" will be seen to be smaller in the boiled crab meat perpared by "low temperature boiling method" than in that by "high temperature boiling method," and also smaller in the bolied crab meat made from unfresh raw meat than in that from fresh raw meat. That is to say, the samller the value of "t_{15}" or "t_{12}", the shorter the time required to reach to the freshness limit.

The case in which the boiled meat was left at 13°C was examined, but it was difficult to determine accurately the values of "t_{15}" or "t_{12}", owing to the fact that the temperature was higher than 3°C.

According to the results obtained by the present author[1,3] or Kaneko[7] who have practical experience in the processing of the canned crab, there is an equation (4) between the storing temperature, "θ", and the time required to reach the decomposition, "t_θ".

$$\log t_\theta = \alpha - \beta\theta \quad \cdots\cdots\cdots\cdots (4)$$

Here, α and β are constants.

Relation between the storing temperature, "θ", and the time, "$\log t_\theta$", shows linear. Also there is the following equation between two storing temperatures, θ_1 and θ_2.

$$\log \frac{t_{\theta_2}}{t_{\theta_1}} = \frac{\theta_2 - \theta_1}{10} \log Q_{10} \quad \cdots\cdots\cdots\cdots (5)$$

Here, "t_{θ_1}" and "t_{θ_2}" are the times required to reach the decomposition stage at the temperatures θ_1 and θ_2 respectively. "Q_{10}" is temperature coefficient. Therefore, if θ_1=3°C and θ_2=13°C, θ_2—θ_1=10°C, then equation (5) becomes briefly $\frac{t_{\theta_2}}{t_{\theta_1}} = Q_{10} \cdots\cdots\cdots (6)$

The values of "Q_{10}" are obtained being 2.3 (mean value) from Table 17.

If the value of "t_{θ_1}" at 3°C (t_{15} or t_{12} at 3°C) is known from Table 17, then the value of "t_{θ_2}" at 13°C ("t_{15}" or "t_{12}" at 13°C) will be obtained.

From each value of "t_θ" obtained, the relation between "$\log t_\theta$" and "θ" is shown being linear as in Fig. 7.

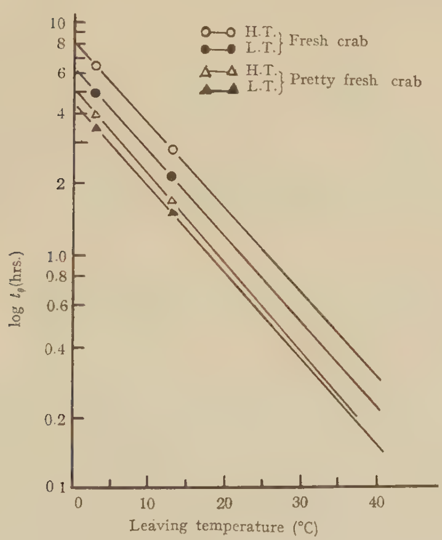
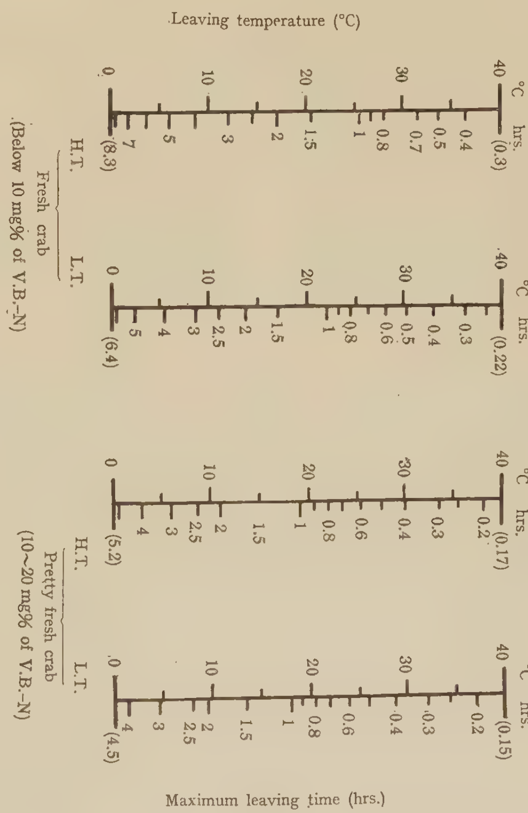


Fig. 7. Relation between the storing temperature "θ" and the time "$\log t_\theta$"

Fig. 8. A scale showing the relation between storing temperature and the limit of freshness in the boiled crab meat



(Fig. 8.)

From Fig. 7, the relation between storing temperature and the limit freshness in the boiled crab meat prepared by each boiling method from the raw crab meat having various freshness is shown as a scale in Fig. 8.

According to Fig. 8, for example, if fresh raw crab meat with the crust (V.B.-N of the raw meat is below 10 *mg*%) is boiled according to "high temperature boiling method" and left at 15°C, the time required to reach the limit freshness will be 2.4 hours. If pretty unfresh raw crab meat with the crust, (V.B.-N of the raw meat is 10∼20 *mg*%) is boiled according to "high temperature boiling method" and left at the same temperature, the time will be 1.4 hours. If the pretty unfresh carb meat is boiled according to "low temperature boiling method" and left at the same temperature, the time will be 1.3 hours. That is to say, it is easily known within what storing time the respective lots of crab meat should be processed.

The maximum storing times obtained by the present author are seen to be pretty too severs; but in the actual procedures, the freshness of each raw crab body is different, so some safety factor should be added. For example, the raw crab bodies in the lower layer of a shipment fall rapidly in freshness.

IV. Calculation of processing time at different temperature for various degrees of freshness of raw or boiled crab meat

It is clear that the degree of freshness of raw crab influences the quality of the canned crab as above described. That degree has some influences not only upon the quality, but also upon the effects of the processing. The determination of the processing time for canned foods according to the freshness of the raw material or boiled meat is very important. The author has studied the calculation of the processing time of canned crab using the same formula as that for canned salmon.[8)]

The important difference of canned crab from canned salmon is the boiling of the raw crab meat with the crust before the filling into cans, in order to remove the meat easily from the crust.

It was described as above that there are two methods for the boiling of the raw crab carcasses. By the high temperature boiling procedures, the actions of enzymes and bacteria are prevented, but the freshness will fall with the elongation of the storing period of the meat boiled at higher temperatures. Therefore the number of bacteria in the meat filled into cans before the processing, varies according to the storing time and temperature. The bacteria are considered to have attached to the raw meat and to have survived in the boiled meat even after the boiling and then to have multiplied. Therefore, those bacteria are considered to be thermotolerant. The kind and the number of the bacteria which survived are different according to the boiling methods, and the storing conditions.

Therefore, the processing time should be controlled in connection with the boiling methods and the storing conditions (temperature and time).

Determination was first made of the upper and lower limits of the processing

temperature, then the processing time was calculated on the basis of the number of bacteria attached to the raw and boiled crab meats between the two limits.

1. The highest processing temperature for the canned crab

The highest processing temperature varies by the freshness of the raw material. For example, when the unfresh raw crab meat is processed at the same highest temperature as fresh raw crab meat, the color and odour of the canned product will become worse and have smell of burning. Here, the author has determined the highest processing temperature for the boiled crab meat treated by "high temperature boiling method", which was left for various storing times at different temperatures, and then processed to the canned crab.

(1) Experimental method

From *Paralithodes camtschatica* bodies captured off Nemuro, Hokkaido, and was landed, the carapace was removed immediately after the landing.

The carcases were boiled at 100°C in sea water, and were left with the crust as follows: (1) left in cool place (2°~10°C) over one night (this is called fresh raw meat; V.B.-N was 6~10 *mg*%), (2) left at the same temperature over two nights (this is called pretty unfresh raw meat; V.B.-N, 15~20 *mg*%), (3) left at the same temperature over three nights (this is called unfresh raw meat: V.B.-N 25~30 *mg*%). Those samples having various degrees of freshness were packed in 1/2 pound flat cans, and were processed at different temperatures (100°~115°C) for different processing times (50, 70, 90 minutes, repectively). The finished sample cans were brought to the laboratory, and then opened. The qualities of the cans were examined both organoleptically and chemically (pH, V.B.-N), and the relation between the freshness of the raw material and processing conditions (temperature and time) was determined and the highest processing temperature for the canned crab was decided.

(2) Experimental results

Results obtained are shown in Tables 19~21.

The quality of the canned crab in Table 21, which was processed from the unfresh raw material (V.B.-N 25~30 *mg*%) was inferior in taste, color and odour. This raw matarial was over the limit of freshness of raw material for canning. The quality of the canned crab in Table 20, processed from pretty unfresh raw material (V.B.-N, 15~20 *mg*%) showed that it is merchantable. The quality of the canned crab in Table 19 processed from the fresh raw material (V.B.-N, 6~10 *mg*%) was good. Commercial canned crab is generally processed from such fresh raw crab.

The influences of the processing factors (temperature-time) upon the quality of the canned crab having different degrees of freshness of the raw material are observed also in Tables 19~21. In the canned crab processed from fresh raw material shown in Table 19, with the rising of the processing temperature, the amount of V.B.-N increased proportionally from the initial amount of V.B.-N in the raw meat. But above 112.7°C (8 pound pressure), the amount of V.B.-N in the canned crab meat showed converse tendency, rather decreased.

Table 19. Quality of the canned crab processed from the fresh raw material (V.B.-N, 6～10 *mg*%)

Processing temp. (°C) (or pressure)	Processing time (mins.)	Quality of canned product							
		V. B. -N in meat (*mg*%)	pH of meat	Color of juice	Colour of meat: Red meat of surface part	Colour of meat: White meat of inner part	Elasticity of meat	Flavour	Isolation of bacteria
105.3°C (3 *lbs*-press.)	50	19.0	6.8	Slightly bluishbrown	Light pinkish	White opaque	Good	Good	+
	70	24.1	6.7	〃	〃	〃	〃	〃	+
	90	26.4	6.7	〃	〃	〃	〃	〃	−
106.9°C (4 *lbs*-press.)	50	21.3	6.7	〃	〃	〃	〃	〃	+
	70	26.7	7.1	Bluishbrown	〃	〃	〃	〃	−
	90	29.9	6.9	Slightly bluishbrown	〃	〃	〃	〃	−
108.4°C (5 *lbs*-press.)	50	20.6	6.8	〃	〃	〃	〃	〃	−
	70	23.9	6.9	〃	〃	〃	〃	〃	−
	90	30.5	6.9	〃	〃	〃	〃	〃	−
109.9°C (6 *lbs*-press.)	50	21.8	6.7	〃	〃	〃	〃	〃	−
	70	26.2	6.9	Slightly violetish and turbid	〃	〃	〃	〃	−
	90	32.3	7.0	Slightly bluishbrown	〃	〃	〃	〃	−
111.3°C (7 *lbs*-press.)	30	20.6	6.8	〃	〃	〃	〃	〃	−
	50	26.7	6.8	Sligitly violetish	〃	〃	〃	〃	−
	70	22.3	6.8	〃	〃	〃	〃	〃	−
112.7°C (8 *lbs*-press.)	30	19.5	6.7	〃	〃	〃	〃	〃	−
	50	25.1	6.8	Strongly violet	Slightly discolor	Slightly browned	Slightly softened	Slightly burned smell	−
	70	26.5	7.1	〃	〃	〃	Softened	〃	−
115.2°C (10 *lbs*-press.)	30	23.6	6.7	Slightly violetish	Faded noticeably	〃	〃	Remarkably burned smell	−
	50	25.6	6.7	Violetish	〃	〃	〃	〃	−
	70	38.0	7.2	〃	〃	〃	〃	〃	−

Table 20. Quality of the canned crab processed from the pretty unfresh material (V.B.-N, 15～20 *mg*%)

Processing temp. (°C) (or pressure)	Processing time (mins.)	Quality of canned product							
		V. B. -N in meat (*mg*%)	pH of meat	Color of juice	Color of meat		Elasticity of meat	Flavour	Isolation of bacteria
					Red meat of surface part	White meat of inner part			
105.5°C (3 *lbs*-press.)	50	25.1	6.6	Slightly bluishbrown	Light pinkish	White opaque	Good	Good	+
	70	25.1	6.7	〃	〃	〃	〃	〃	+
	90	25.9	6.7	Slightly violetish	〃	〃	〃	〃	−
106.9°C (4 *lbs*-press.)	50	26.8	7.0	Slightly bluishbrown	〃	〃	〃	〃	+
	70	25.9	6.7	〃	〃	〃	〃	〃	+
	90	27.1	6.7	Slightly violetish	〃	〃	〃	〃	−
108.4°C (5 *lbs*-press.)	50	30.1	6.7	〃	〃	〃	〃	〃	+
	70	25.3	6.8	Violetish	〃	〃	〃	〃	−
	90	32.1	6.9	〃	〃	〃	〃	〃	−
109.9°C (6 *lbs*-press.)	50	25.6	6.9	Slightly violetish	〃	〃	Slightly softened	〃	−
	70	29.3	7.0	〃	〃	〃	〃	〃	−
	90	32.1	6.9	Violetish	〃	〃	〃	〃	−
111.3°C (7 *lbs*-press.)	30	29.7	6.9	Slightly bluishbrown	〃	〃	Good	〃	−
	50	27.0	6.7	Slightly violetish	Discolor slightly	Slightly brown	Softened	〃	−
	70	37.0	7.0	Violetish	Discolor noticeably	〃	〃	Slightly burned smell	−
112.7°C (8 *lbs*-press.)	30	18.5	6.7	Slightly bluishbrown	Discolor slightly	〃	〃	〃	−
	50	26.2	6.7	Slightly violetish	Discolour noticeably	〃	〃	〃	−
	70	28.4	7.1	Violetish	〃	〃	〃	Remarkably burned smell	−
115.2°C (10 *lbs*-press.)	30	25.2	6.7	Slightly bluishbrown	〃	〃	〃	〃	−
	50	26.9	6.8	Slightly violetish	〃	〃	〃	〃	−
	70	33.4	7.2	Violetish	〃	〃	〃	〃	−

Table 21. Quality of the canned crab processed from the unfresh raw material (V.B.-N, 25～30 $mg\%$)

Processing temp. (°C) (or pressure)	Processing time (mins.)	Quality of canned product							
		V. B.-N in meat ($mg\%$)	pH of meat	Color of juice	Color of meat		Elasticity of meat	Flavour	Isolation of bacteria
					Red meat of surface part	White meat of inner part			
105.3°C (3 *lbs*-press.)	50	28.2	6.8	Slightly pinkish	Light pinkish	White opaque	Good	Slightly NH_3-smell	+
	70	31.5	6.8	〃	〃	〃	〃	〃	+
	90	31.8	6.9	〃	〃	〃	〃	〃	+
106.9°C (4 *lbs*-press.)	50	31.5	6.9	Dark bluish-brown	〃	〃	〃	〃	+
	70	32.3	6.8	Slightly Violetish	〃	〃	〃	〃	+
	90	32.8	6.9	〃	〃	〃	〃	〃	−
108.4°C (5 *lbs*-press.)	50	27.7	7.0	〃	〃	〃	〃	〃	+
	70	30.5	6.9	〃	Dark pinkish	〃	〃	〃	−
	90	35.6	7.1	〃	〃	〃	〃	〃	−
109.9°C (6 *lbs*-peess.)	50	28.4	7.0	〃	〃	Slightly brown	Slightly softened	〃	−
	70	35.2	7.1	〃	〃	〃	〃	〃	−
	90	30.1	7.1	〃	〃	〃	〃	Slightly burned smell	−
111.3°C (7 *lbs*-press.)	30	30.8	6.8	Slightly pinkish	Discolored slightly	〃	Softened	〃	−
	50	33.5	6.7	〃	〃	〃	〃	〃	−
	70	30.1	7.0	〃	〃	〃	〃	〃	−
112.7°C (8 *lbs*-press.)	30	30.9	6.8	〃	Discolored noticeably	〃	〃	〃	−
	50	32.5	6.8	〃	〃	〃	〃	Remarkably burned smell	−
	70	34.9	7.2	〃	〃	〃	〃	〃	−
115.2°C (10 *lbs*-press.)	30	24.9	6.9	〃	〃	〃	〃	〃	−
	50	35.3	7.0	Slightly dark reddish	〃	〃	〃	〃	−
	70	31.6	7.2	〃	〃	〃	〃	〃	−

The pH value also showed movement to alkaline side with the rising of the processing temperature. In organoleptic tests, when the processing temperature was 112.7°C (8 pound pressure), the color and taste of the canned crab were normal when treated by the processing of 30 minutes. But in case of over 50 minutes' processing at the same temperature, the color of the meat became not good, and had some burned smell. The meat softened. The merchantable quality was inferior.

When the processing time was over 70 minutes, the quality was inferior to that treated for 50 minutes. When the processing temperature was 115.2°C (10 pound pressure), the quality of the canned crab was inferior in both color and taste even treated for 30 minutes. The qualities of the cans treated for 50 and 70 minutes both were inferior.

From those results, the upper limit of the processing temperature is considered to be 112.7°C (8 pound pressure), when the raw material is fresh and when the processing time is short (30 minutes). But when the time is over 50 minutes, the processing temperature should be lower.

From the results obtained as above, when the raw meat is fresh, the upper limit of the processing temperature should be 112.7°C (8 pound pressure).

When the pretty unfresh raw crab meat (V.B.-N, 15～20 *mg*%) was processed, the amount of V.B.-N in the meat heated up to 112.7°C (8 pound pressure) increased with the rising of the processing temperature, but at 115.2°C (10 pound pressure) the amount of V.B.-N reversely decreased. The meat heated at 112.7°C showed discoloring and inferior taste and had some burned smell. The meat heated at 115.2°C (10 pound pressure) showed browning of the white meat of the crab, and smelled burned.

From those results, when the raw meat is pretty unfresh, the processing temperature should be below 112.7°C (8 pound pressure).

In the case of the unfresh raw crab meat (V.B.-N 25～30 *mg*%), the increasing amount of V.B.-N was observed as well as in the case of summarized Tables 19 and 20. The unfresh meat heated at 110°C (6 pound pressure) showed already decrease of the amount of V.B.-N. And the red color of the epidermis of crab meat heated over 110°C (6 pound pressure) became dark, while the white meat became brown. When the processing temperature was over 112.7°C (8 pound pressure), the meat acquired a burned smell. This is because that the hydrating ability of the meat became weak with the falling of the freshness during the storage period, consequently the meat was dehydrated. When such dehydrated meat was heated at higher temperature, the meat began to smell burned. From those results, the highest processing temperature of the unfresh raw material should be lower than that of fresh raw material.

According to the bacteriological consideration, the unfresh meat should be processed at higher temperature in order to sterilize the bacteria attached to the content of the cans. But when the unfresh raw material is processed at comparatively higher temperature for longer period, the quality of the canned crab becomes reversely worse as above described. Thus the upper limit of the processing temperature should be varied according to the

different degrees of the freshness of the raw material.

2. The lowest processing temperature of the canned crab

The lowest permissible processing temperature of the canned crab should be the temperature necessary to sterilize the content in the can. Canned crab has no bone to soften, differing from canned salmon. So, the lower the processing temperature, the better the taste and the color of the canned crab. But when the processing temperature is low, the processing time may be too long for economic procedure. From actual technical experience, the lowest temperature is 107°C (4 pound pressure). When the processing temperature is so lower as pressure is 4 pound, the time for maintenance of the temperature of the center of the can will be too long properly to sterilize the content in the can.

Recently in canned crab processed from crab captured by a trawler net, the thermotolerant bacteria which survived after the processing at 110°C were isolated[9]. If such thermotolerant bacteria are often found, the lowest processing temperature should be raised.

3. Calculation of processing time of canned crab having different degrees of freshness

If the highest and the lowest processing temperatures are determined, then for the sake of economical operation, determination must be made of the time required to sterilize the bacteria in the canned crab at the minimum reasonable processing temperature. If the number of bacteria attached to the boiled crab meat increases with the elongation of the storing time, then the processing time should be prolonged, when processing is being done at the same temperature at which the fresh boiled meat is being processed.

It is conveninent for the technologists in canneries that considering those factors which influence the processing, the adequate processing temperature be determined, then that the processing time corresponding to the temperature be determined, and that those relations be showed in simple scales or tables. The author has tried to draw up such scales by studying the relation among the number of bacteria attached to the raw or boiled crab meat, the freshness, and the processing time similar to that has been done in the case of canned salmon[8].

4. The relationship among storing temperature of raw crab meat with the crust, freshness (the amount of V.B.-N produced) and variation of number of bacteria attached

(1) The velocity of decomposition of raw crab meat in the initial stage of storage

In order to exaplain the decomposition velocity of raw crab meat in the initial stage (in V.B.-N production curve), the author has used the same equation as in the case of raw salmon meat[8].

$$V = pt^2 + V_0 \quad \cdots\cdots\cdots\cdots (7)$$

Here, "V" is the amount of V.B.-N in raw meat after "t" hours, and "V_o" is the initial amount of V.B.-N in the raw crab meat when it is carried into a cannery, "p" is the coefficient of the decomposing velocity in the initial stage. Data of V.B.-N produced which were obtained by leaving raw crab meat (with crust) at various temperatures in another experiments were substituted in equation (7), and values of "p" at various temperatures were calculated. The relation between "p" and storing temperatures "t" is shown in Table 22 and Fig. 9.

Table 22. Relation between the storing temperature and velocity of decomposition of raw crab meat with crust in the initial stage of storage

Leaving temp. (°C)	12	17	27	37	47
"p"	0.0039	0.0143	0.0435	0.0983	0.1182

Next, the value of "V_o" (the amount of V.B.-N at "t"=0) and values of "p" were substituted in equation (1); and then the values of "V" (the amount of V.B.-N produced at various temperatures for various periods of time) were calculated. The values obtained here are called $V_{calc.}$ The obtained values of "V" by practical estimation of acutal materials are called $V_{interp.}$ The values of both $V_{calc.}$ and $V_{interp.}$ were compared as in Table 23.

As seen in Table 23, the formation of V.B.-N up to the amount of 30 *mg*%, was found to follow equation (7), because the values of $V_{calc.}$ were almost the same as values of $V_{interp.}$

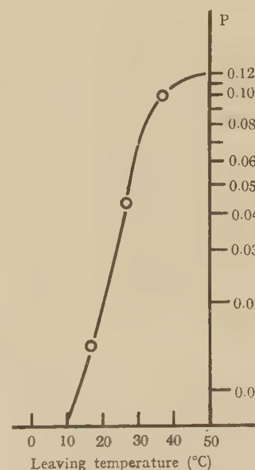


Fig. 9. Relation between the coefficient of the decomposing velocity in the initial stage "p" and storing temperature "t" of raw crab with crust

Table 23. Relation between the values of "$V_{calc.}$" and "$V_{interp.}$"

Leaving temp (°C) / V. B.-N (*mg*%) / Leaving tmie (hrs.)	12		17		27		37		47	
	$V_{calc.}$	$V_{interp.}$	$V_{cal.}$	$V_{interp.}$	$V_{calc.}$	$V_{interp.}$	$V_{calc.}$	$V_{interp.}$	$V_{calc.}$	$V_{interp.}$
0	12.9	12.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9
5	—	—	12.3	12.2	14.1	14.2	14.4	16.5	14.8	15.8
10	—	—	13.3	14.1	16.3	16.1	21.7	18.3	23.7	20.3
20	—	—	13.7	14.9	17.3	20.0	24.2	23.9	26.8	29.4
25		—	17.6	16.5	29.3	20.2	31.1	31.4	59.5	48.9
27	16.8	18.4	20.8	18.9	38.1	25.3	—	—	—	—
30	—	—	24.8	24.0	—	—	—	—	—	—
35	—	—	29.5	31.7	—	—	—	—		—
40	—	—	—	—	—	—	—		—	—
52	23.5	21.9	—	—	—	—	—	—	—	—

(2) Relation between the velocity of decomposition of raw crab meat in the initial stage and the storing temperature-time

In order to show the relation between the velocity of decomposition of raw crab meat in the initial stage and the storing temperature-time, the author has reformed equation (7) as he also did in the case of raw salmon meat[8].

$$pt^2 = V - V_o \quad \cdots\cdots(8)$$

$$\log p + 2\log t = \log(V - V_o) \quad \cdots\cdots(9)$$

$$P_{(p)} + T_{(t)} = V_{(v)} \quad \cdots\cdots(10)$$

Here, the value of $(V-V_o)$ is restricted $0 \leqq V-V_o \leqq 30$, because the value of "V" hasbee restricted up to the value 30 $mg\%$ was reached. From the experimental results obtained previously, "t", the time required to reach 30 $mg\%$ of the amount of V.B.-N, was 50~60 hours, therefore the range of "t" is $1 \leqq t \leqq 60$. The value of "p" was below 0.12 at 47°C of the leaving temperature (see Table 22), because the leaving temperature of raw crab meat is not considered to be 47°C. Therefore the range of the value of "p" is considered to be $0.01 \leqq p \leqq 0.12$.

(3) Making of scale showing the relation between the freshness of the raw crab meat and the storing temperature-time

In order to make a scale showing the relation between the leaving temperature-time and the freshness of raw crab meat, ratios of scales of "p", "t" and "$V-V_o$" of 10 cm length were calculated. As the range of "p" is $0.01 \leqq p \leqq 0.12$ $(1 \leqq 100p \leqq 12)$ and the range of "t" is $1 \leqq t \leqq 60$, ratios of scales of "p" and "t" must be obtained from equation (9).

As "p"-sclae is $\log 12 - \log 1 = 1.2079$, "$t$"-scale is $2\log 60 - 2\log 1 = 1.7782 \times 2 = 3.5564$. In order to make the length of "p" and "t" scale, each 10 cm, scale cofficients, m and n will be obtained as follows:

$$m = \frac{10}{1.2072} = 8.29, \quad n = \frac{10}{3.5564} = 2.82$$

The scale coefficients of "V" are obtained as follows.

$$l = \frac{mn}{m+n} = \frac{8.29 \times 2.82}{8.29 + 2.82} = \frac{23.4}{11.11} = 2.1$$

Here, scales of "p", "t" and "$V-V_o$" are drawn as follows:

For sacle of "p", $x = 8.29\{\log 100p\}$ ············(11)

provided that $0.01 \leqq p \leqq 0.12$

For scale of "t", $y = 2.82\{2\log t\}$ ················(12)

provided that $1 \leqq t \leqq 60$

For sclae of "$V-V_0$", $z = 2.10\{\log 100(V-V_0)\}$ ······(13)

provided that $1 \leqq V\text{-}V_o \leqq 30$

[The distance between the scale of "p" and the scale of "$V\text{-}V_o$"] : [The distance between the scale of "$V\text{-}V_o$" and the scale of "t"] $=m : n=8.29 : 2.82=3 : 1$.

Here, if any values in the ranges of "p", "t" and "$V\text{-}V_o$" respectively are substituted in equations (11) (12) (13), the values of "x", "y" and "z" are shown in Table 24 and Fig. 10.

Table 24. Readings "x", "z" and "y" which are correlated with the values of "p", "v" and "t" on scales "p", "$V-V_0$" and "t"

P-scale		$(V-V_0)$-scale		t-scale	
p	x (cm)	v (mg%)	z (cm)	t (hrs.)	y (cm)
0.01	0	1.0	2.10	1	0
0.02	2.49	2.5	2.94	5	3.94
0.03	3.95	5.0	3.55	10	5.64
0.04	4.98	7.5	3.94	15	6.64
0.05	5.80	10.0	4.20	20	7.33
0.06	6.45	12.5	5.04	25	7.88
0.07	7.00	15.0	5.67	30	8.34
0.08	7.50	17.5	6.82	35	8.71
0.09	7.91	20.0	6.94	40	9.05
0.10	8.92	25.2	7.12	45	9.32
0.11	8.63	30.0	7.31	50	9.58
0.12	8.95	—	—	55	9.82
—	—	—	—	60	10.00

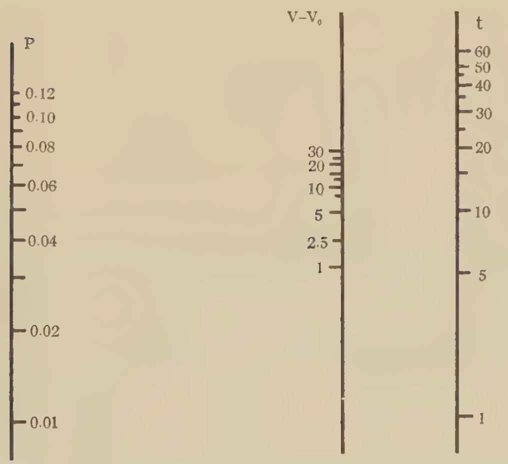


Fig. 10. Scales showing "p", "$V\text{-}V_0$" and "t"

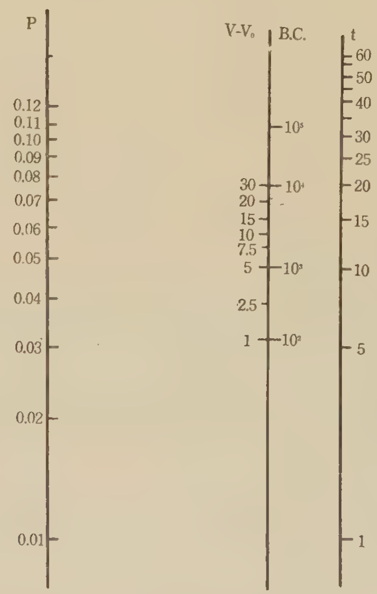


Fig. 11. Scales showing "p", "$V\text{-}V_0$," "t" and "$B.C.$" (bacterial counts)

(4) Relation between the increase of the amount of V.B.-N and increase of the bacterial counts with the falling of freshness

From the following Table 25 (p. 123), the bacterial counts (*B.C.*) are drawn to the right side of the scale "$V-V_0$" in Fig. 11.

(5) Relation between the leaving temperature-time and the bacterial counts

Here, Figs. 9, 10 and 11 are together drawn as shown in Fig. 12.

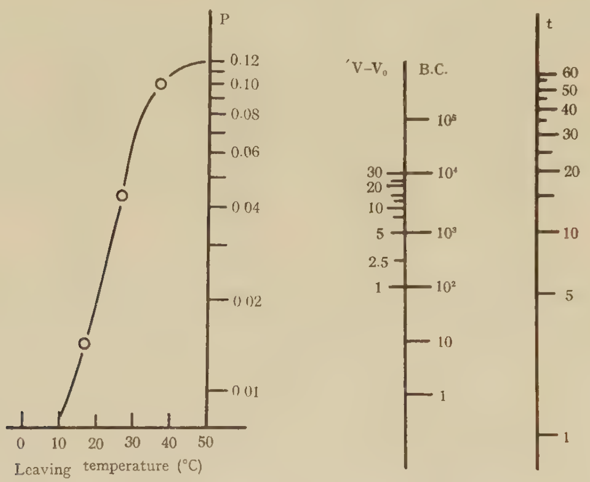


Fig. 12. Diagrams showing the relation among the leaving temperature-time and the bacterial counts and V.B.-N

If the leaving temperature of the raw crab meat in a cannery is known, crose the perpendicular line corresponding to the given temperature and the hyperbolic line showing the relation between the coefficient of the decomposing velocity in the initial stage and the leaving temperature. From the point of the crossing, a horizontal line is drawn and is crossed with "p"-scale. The crossed point is combined with any leaving time on "t"-scale by a straight line. The point at which the straight line and "$V-V_0$"-scale cross, shows the increase of the amount of V.B.-N on the left side and the bacterial counts on the right side of "*B.C.*"-scale.

5. The variation of the amounts of V.B.-N and bacterial counts during the storage of the boiled crab meat

(1) Varations of the amounts of V.B.-N and bacterial counts during the procedure of the boiling of the carcasses of crab

In the procedure of crab canning, the carcasses from which the carapaces were removed

are boiled with the crust by "high-" or "low temperature boiling method". After the boiling at high temperature, the carcasses are cooled in water, and then the crusts are split off by a knife in order to take out the meat.

The bolied crab meat treated by "low temperature boiling method" is pushed out by a pressing-roll or by water pressure. The quality of the canned crab, *e.g.*, color, or hardness of the meat, is influenced largely by the conditions of the boiling of the carcasses. Therefore the boiling is an important procedure.

During the boiling, the meat in the crust is dehydrated, the chemical components flow out with the water, and the amount of V.B.-N and number of bacteria in the meat vary. Those variations are influenced by the conditions of the boiling (*e.g.*, boiling methods, temperature and time).

The variations of the amount of V.B.-N and the number of bacteria in the boiled crab meat treated by "high temperature boiling method" (100°C at 18 minutes), or treated by "low temperature boiling method" (60°C at 10 minutes) are shown in Table 25.

Table 25. Variations in the amounts of V.B.-N and the bacterial counts of crab before and after boiled by the "high" and "low temperature boiling methods"

Items / Boiling method	Raw meat			Boiled meat		
	pH	V.B.-N (*mg*%)	Bacterial counts per *g*	pH	V.B.-N (*mg*%)	Bacterial counts per *g*
High temp. boiling method	6.6	6.3	5×10^2	6.7	10.9	2.5×10^2
	6.2	16.7	2×10^3	6.4	12.0	1×10^3
	6.4	25.0	5×10^3	6.4	7.1	2×10^3
	6.8	33.7	1×10^4	6.8	9.3	8×10^3
Low temp. boiling method	6.6	6.3	5×10^2	6.3	10.1	3.3×10^2
	6.2	16.7	2×10^3	6.4	10.4	1.5×10^3
	6.4	25.0	5×10^3	6.4	8.2	3×10^3
	6.8	33.7	1×10^4	6.8	14.2	1×10^4

As seen in Table 25, the amount of V.B.-N and the number of bacteria decrease in the crab meat during the boiling procedure. The degree of the decrease is influenced by boiling methods or by the degrees of freshness of the raw crab meat. The decrease of the amount of V.B.-N and the number of the bacteria in the boiled crab meat treated by the "high temperature boiling method" was remarkably more than those by the "low temperature boiling method".

As to the decrease of number of bacteria in the boiled crab meat, according to freshness of raw crab meat, the ratio of the decrease of the boiled meat prepared from the raw meat of which the amount of V.B.-N is below 20 *mg*% (the limit of the freshness) is about 50 % when it is treated by the "high temperature boiling method" and about 30% when treated by "low temperature boiling method". But the ratio of the decrease in the boiled meat prepared from the raw meat of which the amount of V.B.-N is above 20 *mg*% is slight.

This may be due to the fact that with the falling of the freshness, the number of bacteria increases, and the bacteria make the spores, and the thermotolerance increase.

(2) Increase of the amount of V.B.-N and the number of bacteria during the storage of the boiled crab meat

The once decreased amount of V.B.-N and number of bacteria by the boiling procedure both increase gradually again during the storage of the boiled crab meat. The rates of the increase are different in accordance with the boiling methods.

The rate of the increase in the boiled meat treated by "low temperature boiling method" is more remarkable than in that treated by "high temperature boiling method."

The rate of increase in the amount of V.B.-N and the number of bacteria at the higher leaving temperatures are more remarkable. With the elongation of the storage period, that increase maintained.

The relation between the velocity of decomposition (the variation of the amount of V.B.-N and the number of bacteria) of the boiled crab meat treated by "high-" or "low temperature boiling method" and the leaving temperature-time are shown in Table 26 and Figs. 13 and 14.

As seen in Table 26 and Figs. 13 and 14, the increases in the amount of V.B.-N and the number of bacteria in the boiled crab meat were slow in the initial storage period, but after a certain point, the increases were rapid. The increase in the boiled crab meat samples from raw unfresh meat were larger than that from raw fresh meat. Differing from the canned salmon which is processed directly from the raw meat, the processing condition of the canned crab which is processed after the boiling of the raw meat (carcass) may be influenced by the degrees of freshness of the boiled meat as well as the degrees of freshness of the raw

Tables 26. The relation between the variation of the amounts of V.B.-N and the bacterial counts of the boiled crab meat treated by "high" or "low temperature boiling methods" and the leaving temperature-time

Boiling method		High temp. boiling method				Low temp. boiling method			
Temp. (°C)		5		10		5		10	
Sample	Time (min.) \ Freshness	V. B.-N ($mg\%$)	Bacterial counts per g.	V. B.-N ($mg\%$)	Bacterial counts per g.	V. B.-N ($mg\%$)	Bacterial counts per g.	V. B.-N ($mg\%$)	Bacterial counts per g.
Fresh material	2	10.9	2.5×10^2	7.9	1×10^2	10.1	3.3×10^2	10.4	7×10^2
	6	15.8	2.5×10^2	18.2	5×10^2	16.4	5.0×10^2	19.0	2×10^3
	20	16.3	3.5×10^2	19.5	3×10^3	17.7	8.6×10^2	22.6	2×10^4
	40	17.2	2.4×10^3	22.0	3×10^4	22.0	4.5×10^3	27.8	1×10^5
	52	20.2	3.5×10^4	24.6	1×10^5	—	—	30.4	8×10^5
	60	—	6.5×10^4	29.0	5×10^5	50.8	3.5×10^5	—	10^6
Unfresh material	0	9.3	8×10^3	9.3	8×10^3	14.2	1×10^4	14.2	8×10^3
	24	13.4	1×10^4	17.3	2×10^4	14.7	1×10^4	10.3	1×10^4
	48	12.6	1×10^4	19.2	3×10^4	17.8	3×10^4	21.1	2×10^4
	72	12.3	2×10^4	24.6	3×10^5	17.2	8×10^4	25.4	8×10^5
	96	13.7	2×10^4	25.8	1×10^6	23.5	1×10^5	33.6	1×10^6

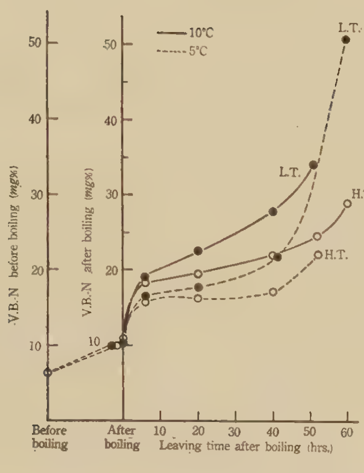


Fig. 13. Variations in the amounts of V.B.-N of the boiled crab meat treated by "high" or "low temperature boiling method" and left at various temperatures and times

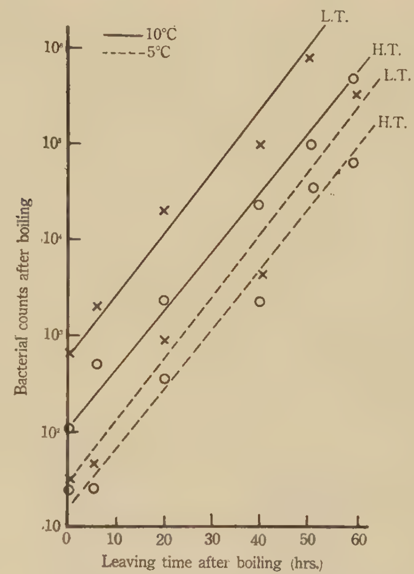


Fig. 14. Variations in the bacterial counts of the boiled crab meat treated by "high" or "low temperature boiling method" and left at various temperatures and times

material.

The limit of the freshness of the boiled crab meat is influenced by the degrees of freshness of the raw material. When the amount of V.B.-N in the raw meat was below 10 *mg*%, the limit of the freshness of the boiled crab meat should be 15 *mg*% of V.B.-N; when the amount of V.B.-N in the raw crab meat was 10~20 *mg*%, the limit of the freshness of the boiled meat should be 10~20 *mg*% of V.B.-N.

If the limit of the freshness of the boiled crab meat is considered on the basis of the number of bacteria in the boiled meat, good quality canned crab is not obtainable unless the number of bacteria is less than $10^2 \sim 10^3$ per *g* in the boiled material. From the experimental results, if various conditions, *e.g.*, the number of bacteria in the raw crab meat before boiling, the boiling methods, the storage temperature-time after the boiling, are known, the number of bacteria in the boiled crab meat can be presumed. Next, if the number of bacteria in the boiled crab meat is known, the conditions of the processing of the canned crab can be calculated.

Here, the consolidated scale shown in Fig. 15, indicates the number of bacteria in the boiled meat, resulting from the number of bacteria in the raw crab meat, the boiling methods, and the storage temperature-time after the boiling.

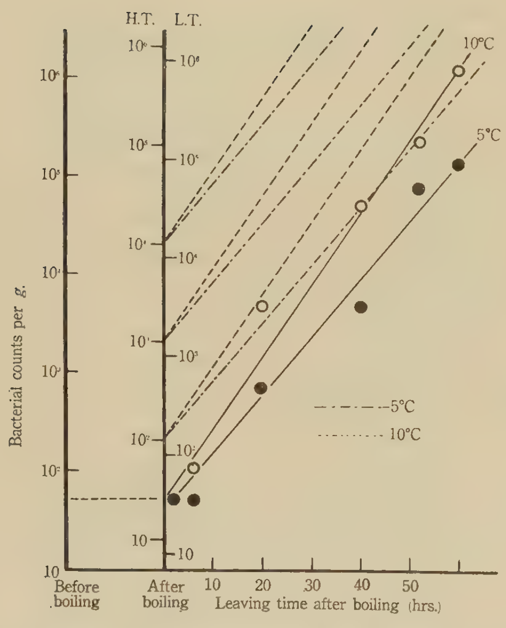


Fig. 15. Diagram showing the number of bacteria in the boiled crab meat, resulting from the number of bacteria in the raw crab meat, the boiling method, and the storage temperature after the boiling

6. *Calculation of processing time, when the boiled crab meat having various degrees of freshness is processed*

(1) Thermotolerance of bacteria attached to the boiled crab meat

The processing conditions depend upon the thermotolerance of bacteria attached to the boiled crab meat.

The author has previously isolated thermotolerant bacteria from the boiled crab meat. The bacteria were identified to be *Bac. subtilis var.* of which the thermotolerance is shown in Table 27.

This *Bac. subtilis var.* has been often isolated from the swelled canned crab with *Bac. megatherium* by the present author[10]. The thermotolerance of *Bac. megatherium* which was also studied by the present author, is shown in Tables 28 and 29.

Table 27. Thermal death time of *Bac. subtilis var.* isolated from boiled crab meat

Heating temp.	Thermal death time (min.)
100°C (212°F)	140
108°C (228°F)	60
110°C (230°F)	30
111°C (232°F)	20
115°C (239°F)	8

Table 28. Thermal death time of *Bac. megatherium* isolated from swelled canned crab

Heating temp.	Thermal death time (min.)
100°C (212°F)	140
105°C (221°F)	60
110°C (230°F)	28
115°C (239°F)	8

Table 29. Number of *Bac. megatherium* survived after the heating at 105°C (221°F)

Heating time (min.)	Number of survived bacteria
0	10,000
10	3,000
20	500
30	130
40	20
50	5

Comparing Table 27 with Tables 28 and 29, no remarkable difference between *Bac. subtilis var.* and *Bac. megatherium* in respect to thermotolerance is observable. Here, as an example of the kind of bacteria in the canned crab, use was made of *Bac. megatherium*, which is a spore-forming bacerium and has been frequently isolated from the swelled canned crab.

(2) Heat-penetrating curve in the processing of canned crab

It is important to know the temperature of the center of the can content during the processing of the canned crab in order properly to calculate the processing time.

Estimations were made of the heat-penetration into the canned crab of 1/2-pound flat can which was processed from the boiled crab meat treated by "high-" or "low temperature boiling method". The heat-penetration was estimated by a thermocouple during the processing at 108.4°C (5 pound pressure), 109.9°C (6 pound pressure), 111.3°C (7 pound pressure) and 112.7°C (8 pound pressure). The heat-penetrating curves (by "high-" or "low temperature boiling method") at 109.9°C (6 pound pressure) are representatively shown in Fig. 16.

From Fig. 16, calculation is made of the value of f_h, that is the time required to reduce the difference between the retort temperature and the temperature at the center of the can content to reach 1/10 value of that original difference. The value of f_h was 47 for the canned crab subjected to the "high temperature boiling method"; the value was 35 for that subjected to the "low temperature boiling method."

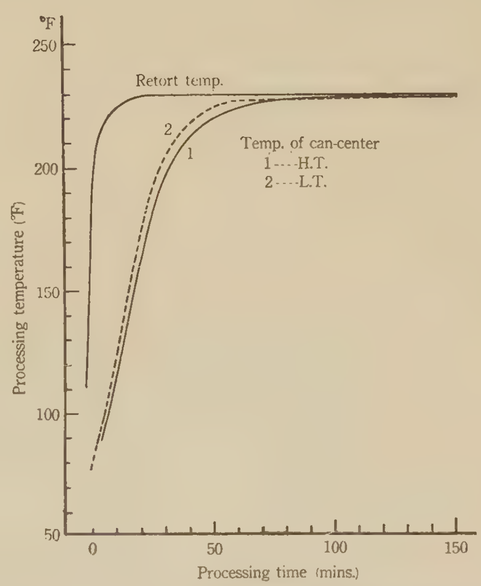


Fig. 16. Heat penetration curves of canned crabs which were prepared by "high" or "low temperature boiling method" at 109.9°C (6 pound pressure)

The heat-penetrating curves are different according to boiling methods of raw crab meat as seen in Fig. 16. The heat-penetration obtained from the canned crab subjected to the "high temperature boiling method" is worse than that subjected to the "low temperature boiling method.". This may be due to the reason that the boiled crab meat treated by the former method has less water-content than that treated by the latter method, because the dehydrating ratio of the former material is larger than that of the latter.

(3) Calculation of the processing time

The processing time was calculated after Stumbo[11] who is considering the number of bacteria before the processing and surviving bacteria. Procedure was the same as in the paper dealing with canned salmon[8].

Stumbo's equations are as follows:

$$U = Z\ (log\ a - \log b) \quad \cdots\cdots\cdots\cdots (14)$$

or $$U = Z\ (log\ a + P) \quad \cdots\cdots\cdots\cdots (15)$$

Here, "U" is the time required to reduce the number of bacteria attached to the content of the canned foods to a definite aimed number of surviving bacteria, "Z" is the inclination of the thermal death rate curve at a definite processing temperature, "a" is initial number of bacteria per unit of the raw material of a canned food, "b" is the number of bacteria in the canned food after the processing. "P" is logarithm of reciprocal of the number of the bacteria after "U" minutes. Here, it is assumed Z=0.5, and z=20.5 ("z" represents the degree of slope of the thermal death time curve.).

Then the processing time was calculated at various processing temperatures (5, 6, 7 and 8 pound pressures) using materials with different degrees of freshness of the boiled crab meat treated by two boiling methods respectively. Here, as an object of the kind of bacteria in the canned crab, use was made of *Bac. megatherium* as above described. The number of bacteria attached to the boiled crab meat was employed from 10^2 to 10^5 per 1 *g*. The value of "b" was taken to be 0.01 of survival number of bacteria in the canned crab.

The results calculated are shown in Table 30 and Fig. 17.

Table 30. Relation between the degrees of freshness of raw crab meat and the processing temperature-time

Bacterial counts / Boiling method / Processing temp.	10^2		10^3		10^4		10^5	
	High-temp.	Low-temp.	High-temp.	Low-temp.	High-temp.	Low-temp.	High-temp.	Low-temp.
108.4°C (227.2°F) (5 *lbs*-press.)	min. 90.8 (108.0)	85.6 (102.8)	98.6 (118.2)	92.6 (111.2)	105.4 (126.7)	99.5 (116.3)	112.5 (135.0)	106.1 (127.4)
109.9°C (229.8°F) (6 *lbs*-press.)	74.4 (89.3)	70.1 (84.2)	79.7 (95.6)	75.2 (90.3)	85.4 (102.3)	80.5 (96.6)	91.9 (110.2)	86.2 (103.3)
111.3°C (232.4°F) (7 *lbs*-press.)	64.6 (77.5)	60.9 (73.0)	68.5 (82.2)	64.6 (77.5)	72.3 (86.8)	68.1 (81.7)	77.8 (93.4)	73.4 (88.1)
112.7°C (234.8°F) (8 *lbs*-press.)	63.2 (75.8)	59.6 (71.5)	65.5 (78.6)	61.7 (74.0)	67.0 (80.5)	63.2 (75.8)	72.6 (87.1)	68.5 (82.2)

In Table 30, parentheses show the processing time to which was added 20% of safety factor[7,8], considering various factors, *e.g.*, velocity of rise in temperature of cans, or the kinds of bacteria attached to the raw material assuming the bacteria to be *Bac. megatherium*.

As seen in Fig 17, the processing time at any definite processing temperature must be increased according to the increase of the number of bacteria in the boiled crab meat. If the number of bacteria attached to the boiled crab meat prepared by both boiling methods is the same, the processing time for the boiled meat treated by the "high temperature boiling method" should be somewhat longer than that treated by the "low temperature boiling method".

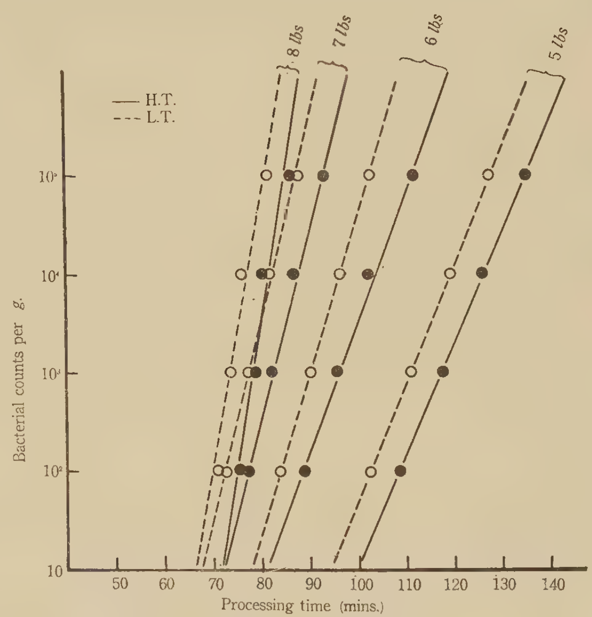


Fig. 17. Relation between the degrees of freshness of raw crab meat and the processing temperature-time

7. *Consolidated scale showing the relations among the storage temperature of the raw or boiled crab meat, boiling methods, falling of freshness and the processing temperature-time of canned crab*

Fig 12, Fig. 15 and Fig. 17 are put together in a consolidated scale as shown in Fig. 18.

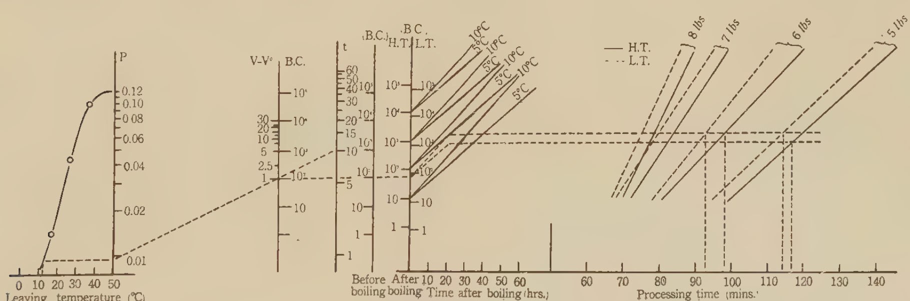


Fig. 18. Consolidated scale showing the relations in canned crab among the storage temperature of the raw or boiled crab meat, boiling methods, falling of freshness and the processing temperature-time

In Fig. 18, if the room temperature in a cannery is 12°C, the coefficient ("*p*") of the decomposing velocity of raw crab meat in the initial stage is known to be 0.009.

Then, if the leaving time of the fresh raw material (V.B.-N, 10 *mg*%) is 10 hours after

unloading into a cannery, the amount of V.B.-N in raw meat shows 11 *mg*% resulting with the increase of 1 *mg*% of V.B.-N, and at that time the number of bacteria is 10^2. Here the raw meat with the crust is boiled by "high-" or "low temperature boiling method. By the boiling procedure, the number of bacteria is descreased to about 7×10^1 or 9×10^1 in the case of "high-" or "low temperature boiling method" respectively. If the boiled crab meat is left at 10°C for 20 hours, the number of bacteria becomes about 1.3×10^3 or 2×10^3 in "high-" or "low temperature boiling method.

Then, the safety processing time is found to be 98 minutes at 6 pound pressure for the boiled meat treated by "high temperature boiling method", and 92 minutes for the boiled meat treated by "low temperature boiling method".

In this paper, a plan of making up consolidated scales for crab canning by which the adequate processing time may be found from the leaving temperature-time of raw and boiled crab meat, was described. The scale may be convenient for the technologists of canneries.

V. Some problems concerned with canned crab processed subjecting to the "low temperature boiling method"

1. *Topics and the explanation*

The "low temperature boiling method" which has been invented by Kosakabe[2] in order to prevent the blueing of the canned crab meat, is being actively employed in the making of canned boneless crab. However, the "low temperature boiling method" involves many problems to be solved by scientific studies, therefore this method is now in a test period. Here, comparing the canned crab subjected to "low temperature boiling method" with that subjected to the "high temperature boiling method", the scientific problems arising from the use of the "low temperature boiling method" will be summarized in a list as shown in Table 31.

As seen in Table 31, in the canned crab which has been processed by the usual method ("high temperature boiling method"), blue meat was often seen, as a result of which the packers have suffered damages, and also the uneatable bone (tendon) is left in the product.

On the other hand, in the canned crab processed by subjecting to the "low temperature boiling method", there are many problems : (1) browning, (2) black spots, (3) decrease of the yield of canned crab meat, (4) inferior taste, and (5) occurrence of crystals, etc.

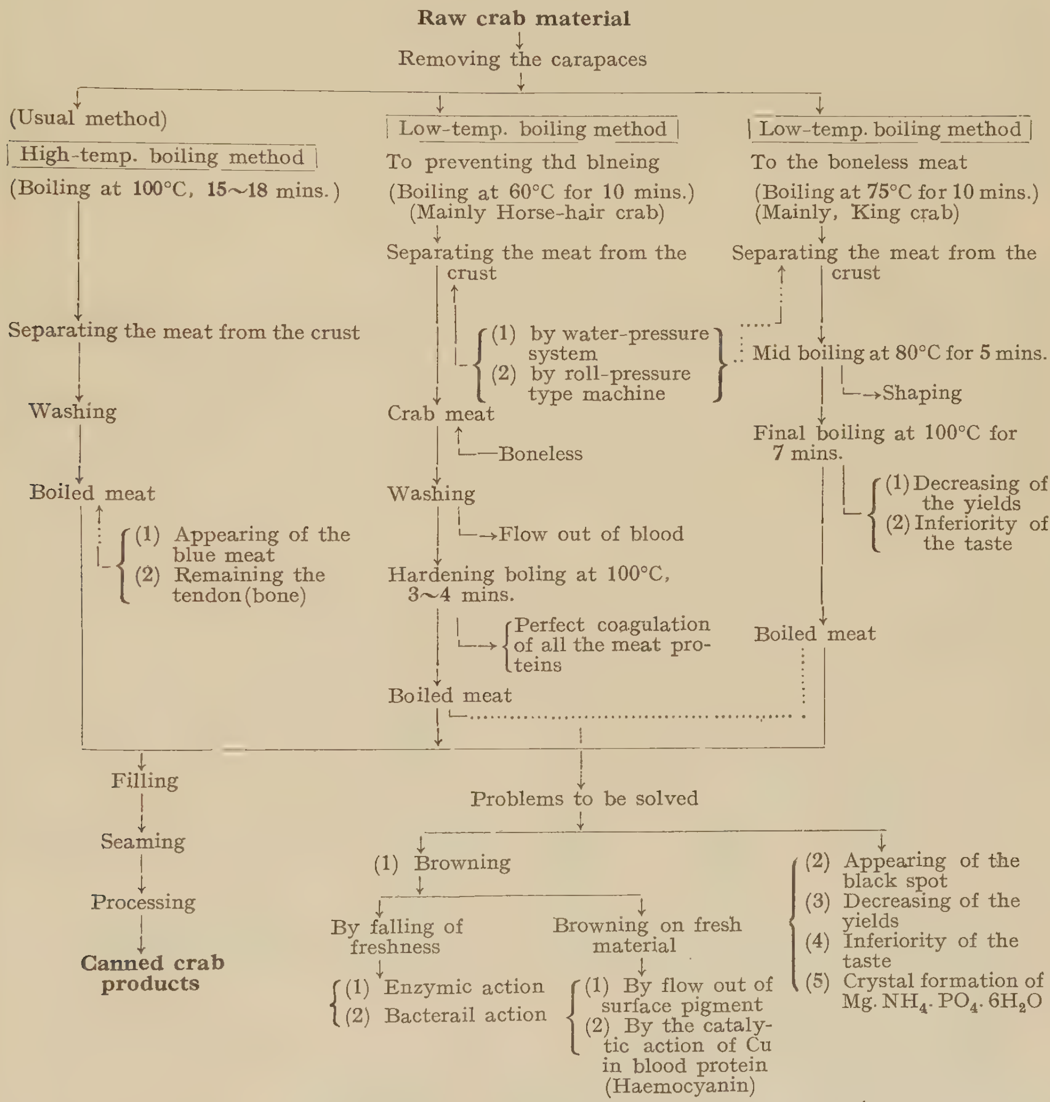
Here, those problems will be discussed on the basis of the authors previous studies.

(1) Browning of the canned crab meat

The browning of canned crab meat means the browning of the white part of the meat. This browning has been observed even in the processed canned crab which was treated by the "high temperature boiling method" from unfresh raw material.

According to Nagasawa[12], a member of the staff of the author's laboratory, the cause of the browning may be found in the use of unfresh raw material. In the canned crab which

Table 31. Some problems concerned with canned crab processed by subjecting to the "low temperature boiling method"



was processed by subjection to the "low temperature boiling method," the falling of the freshness which caused by the action of enzymes remaining after the boiling surely plays a part in the browning. In the same processed canned crab, even when fresh raw material is used, browning often occur. The casue, the mechanism and the methods of preventing the browning are discussed in Nagasawa's paper[12].

(2) Black spots on the canned crab meat

This matter is different from the blackening of the canned crab meat which had been observed in the past, caused by the combination of the metals of tinplate of the container and the hydrogen sulfide produced from the meat during the processing. Such black spots appear near the ends of cut leg meat in the canned crab, especially in *Erimacrus isenbeckii*.

This may be explained by the belief that tyrosinase in the blood of crab decomposes tyrosin in the meat and forms melanin. The details of the results of investigation will be described in another paper.

(3) Taste

It is said that the taste of the canned crab processed from the boiled meat treated by "low temperature boiling method" is inferior to that treated by "high temperature boiling method"[13]. This may be due to the reason that when the boiled meat treated by the former method is filled into cans, the boiled meat has abundant water content, and when it is processed, the water flows out abundantly from the meat as juice accompanying with taste components. Therefore the solid of crab meat itself loses the taste.

The details will be presented in another paper.

(4) The decrease in the amount of solid content of canned crab

When the raw carcasses are boiled by the "low temperature boiling method", the ratio of dehydration of the meat is small. The hardening-boiling after the low temperature boilng is done in short time, so the dehydration is small. Therefore large volume and large water-content are obtained in the boiled meat treated by the "low temperature boiling method" before the filling into the cans. So it is difficult to seal the cover being raised by the meat having a large quantity of water.

But when the filled cans are processed in a retort under high temperature, the meat is dehydrated, the solid content becomes small and a larger amount of the juice is obtained in the can.

This may be also explained by the difference in the ratio of dehydration as formerly described[4].

(5) Formation of crystals

The cause of the formation of glass-like crystals ($Mg \cdot NH_4PO_4 \cdot 6H_2O$) in the canned crab has been cleared already by the present author and Nagasawa[14].

It is said that the crystals are found in the canned crab treated by "low temperature boiling method". This may be due to the reason that there is a larger amount of Mg ion in the free state in the canned crab meat treated by "low temperature boiling method". It has been cleared that the formation of the crystals in the canned crab treated by the "low temperature boiling method" can be prevented by boiling in water containing polyphosphate, "Calgon". The details will described in another paper.

2. *Autolysis of crab meat after the "low temperature boiling"*

Scientific problems arising in the case of canned crab processed from the boiled meat treated by the "low temperature boiling method" are caused not only by the characteristics of the crab meat tissue itself, but also largely by the action of autolytic enzymes after the "low temperature boiling". For example, by autolysis of the crab meat, decomposition is expedited and browning occurs easily. Studies were made on the velocity of the autolysis of the crab meat after the low temperature boiling, also optimum temperature and pH of the autolytic enzyme in the crab meat were determined.

(1) Experimental method

Shoulder and leg meats were taken from fresh raw crab carcasses; each meat was crushed and homogenized. Ten *g* of the homogenized crab meat, 10 cc of distilled water and 5 *cc* of toluene were put into a large sized test tube, shaken and plugged with a rubber stopper. The sample thus made was left at 27°C or 37°C. After a definite duration of the leaving time, the 10 *g* of the meat suspension taken out from the test tube was employed for the estimation of amino acid nitrogen produced.

(2) Experimental result

Results obtained from the use of raw meat are shown in Table 32 and Fig. 19.

Table 32. Variations in the amounts of amino acid nitrogen in raw crab meat during the course of autolysis

Temp. / Parts / Time (hrs.)	27°C		37°C	
	Shoulder meat	Leg meat	Shoulder meat	Leg meat
	mg%	mg%	mg%	mg%
0	224	198	224	224
24	224	205	243	237
48	243	224	378	282
72	326	250	525	352
96	306	358	582	429
144	544	448	646	506
168	621	518	653	570

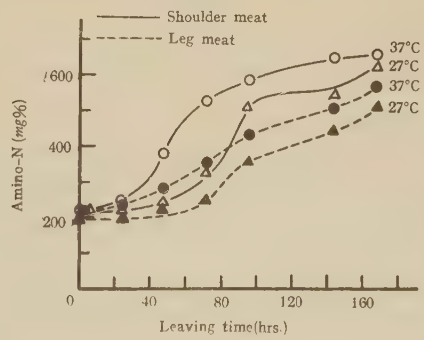


Fig. 19. Variations in the amounts of amino acid nitrogen in raw crab meat during autolysis

After the heating of raw shoulder and leg meats treated by the "low temperature boiling method" at 65°C for 10 minutes, 10 *g* of the boiled meat was treated in the same manner as above described. The results obtained from the use of boiled meat are shown in Table 33 and Fig. 20

Table 33. Variations in the amounts of amino acid nitrogen during autolysis in boiled crab meat treated by "low temperature boiling method"

Time (hrs.) \ Temp. / Parts	0°C		27°C		37°C	
	Shoulder meat	Leg meat	Shoulder meat	Leg meat	Shoulder meat	Leg meat
	mg%	*mg%*	*mg%*	*mg%*	*mg%*	*mg%*
0	160	108	163	106	173	96
24	160	108	166	109	186	102
48	166	122	198	109	275	154
72	—	—	—	—	320	154
96	173	123	218	134	429	294
120	—	—	282	250	525	467
144	192	126	390	326	—	—

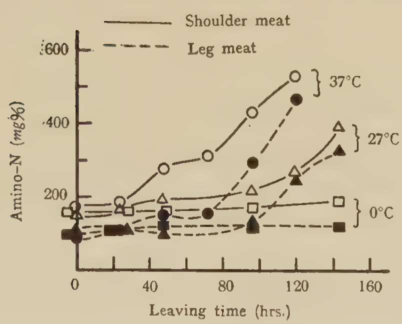


Fig. 20. Variation in the amounts of amino acid nitrogen during the autolysis of boiled crab meat which was treated by "low temperature boiling method"

As seen in Figs. 19 and 20, the autolysis continues to advance even after the "low temperature boiling". With the rising of the leaving temperature, the velocity of the autolysis increases. The velocity of the autolysis of the shoulder meat in raw or boiled state is more rapid than that of leg meat.

Whether the data obtained can be applied to the equation of monomolecular reaction after Oya,[15] or not, has been discussed. After consideration, it was decided that the relation between "t" and "$\log \frac{a}{a-x}$" was apparently linear and that it can be applied to the equation.

$$\log \frac{a}{a-x} = Kt + C \quad \cdots\cdots\cdots\cdots (16)$$

Here, "a" is the total amount of amino acid nitrogen in the sample which was hydrolyzated by conc. HCl solution. "x" is the increased amount of amino acid nitrogen at estimating time, "t"; "K" is velocity constant in the autolysis; "C" is a constant.

The temperature coefficient ("Q_{10}") in the autolysis is determined by equation (17).

$$Q_{10} = \frac{K_{\theta+10}}{K_\theta} \quad \cdots\cdots\cdots\cdots(17)$$

Here, "θ" is the leaving temperature; "K_θ" and "$K_{\theta+10}$" are the values of the velocity constant in the autolysis at "θ°" and "(θ+10)°C". The values of "K" and "Q_{10}" which were calculated from Figs. 19 and 20 are shown in Table 34.

Table 34. Values of "K" and "Q_{10}" in the autolysis of crab meat

Kinds of meat	Meat parts	Velocity constant ($K\times10^6$)		Temperature coefficient
		37°C	27°C	(Q_{10})
Raw meat of *Paralithodes camtschatica*	Shoulder meat Leg meat	980 490	420 200	2.34 2.45
Same as above. (Low temp. boiling method)	Shoulder meat Leg meat	475 200	200 80	2.38 2.50
Erimacrus isenbekii	Mixed meat (Shoulder and Leg)	1,700 (35°C)	590 (25°C)	2.6 (0°~35°C)

In Table 34, the data obtained as to *Erimacrus isenbeckii* meat[1] are also included. According to that table, the following conclusion will derived: (1) The value of "K" (velocity constant of the autolysis) decreased to a half after the "low temperature boiling", but the autolysis did not stop. (2) The velocity of autolysis of leg meat decreased to a half that of the shoulder meat in raw state or the boiled meat treated by the "low temperature boiling method". (3) The values of "Q_{10}" in the raw or the boiled meat showed the same. The mean value of "Q_{10}" for the shoulder or leg meats was about 2.4. (4) The value of "K" of *Erimacrus isenbeckii* meat showed larger than that of *Paralithodes camtschatica* meat. That is to say, the autolytic action of the former is stronger than that of the latter.

From the results as above obtained, when the raw crab carcasses are boiled by the "low temperature boiling method", the autolytic enzymes show activation, especially in shoulder meat, after the boiling. This may be due to the fact that the shoulder meat is incontact with the viscera enzymes.

3. *Optimum temperature for the autolytic enzyme of the crab meat and the enzyme-destroying temperature*

In order to determine the optimum temperature for autolytic enzyme in *Paralithodes camtschatica* meat, the author has estimated the amounts of amino acid nitrogen produced in 10 *g* samples of fresh raw leg meat antiseptized with toluene, which were left at different temperatures for 12 hours.

Results obtained are shown in Table 35 and Fig. 21.

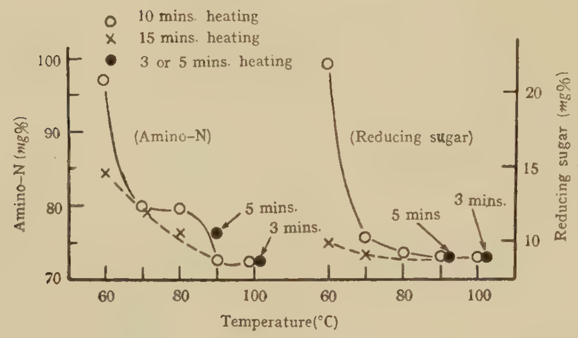


Fig. 22. Heat resistance of autolytic enzymes of crab meat

As seen in Table 36 and Fig. 22, by the heatings of the raw crab meat for 10 or 15 minutes respectively, the amounts of amino acid nitrogen and reducing sugar produced in the meat heated at 60°~90°C decreased, while heating at above 90°C resulted in the amounts of amino acid nitrogen and reducing sugar produced in the heated crab meat before and after the leaving period being almost the same. That is to say, the autolytic action of the crab meat was destroyed by above 90°C heating.

By the "high temperature boiling method" (at 100°C for 15~20 minutes), which has been hitherto employed, the autolytic action in the heated crab meat was completely stopped. Therefore the decomposition of the boiled meat becomes slow.

On the other hand, in case of the "low temperature boiling method", the autolytic action of the boiled meat is maintained until the hardening boiling (100°C, 3~5 minutes), which is done after the "low temperature boiling", as above described. In this case, if the leaving period of the boiled crab meat is comparatively long until the hardening boiling, the decomposition becomes rapid.

VI. Soured smell of canned crab

It has been often observed in canned crab that the meat tastes sour slightly, though the top and bottom of the can are flat. It is called "flat sour" of canned foods, of which the cause is some kinds of thermophiles.

However, studies were made of the "flat sour"-like canned crab, of which the cause was ascertained to be not bacteria. The chemical components of the soured substance have been identified.

1. Chemical components of the soured substance in the soured canned crab meat

Canned crab which was processed in "fancy"-style and was rejected because of "flat sour" upon inspection, was used for the study.

Though the quality of this canned crab was almost the same as that of the normal cans in flesh and juice, it has soured taste. Those canned crab were incubated at 37° or 50°C

Table 35. Relation between the temperature and the amounts of amino acid nitrogen produced during the autolysis of crab meat

Temperature	Amino-N (*mg*%)	Temperature	Amino-N (*mg*%)
0°C	181	70°C	212
25	214	80	196
37	230	90	181
50	242 (Opt.)	Remarks	181 *mg*% in initial sample
60	221		

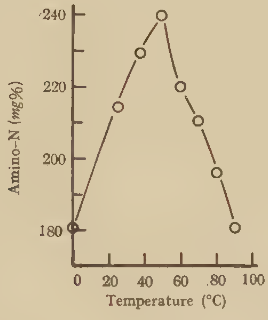


Fig. 21. Relation between the temperature and the amounts of amino acid nitrogen produced during the autolysis of crab meat

As seen in Fig. 21, the autolytic action of the enzyme in the crab meat was maximum at 50°C. If the temperature were over 50°C, the action decreased. Even at a higher temperature, at 80°C, the action was observed slightly.

Next, the raw crab meat was heated at 60°, 70° and 80° for 10 or 15 minutes, or at 90° and 100°C for 5 or 3 minutes respectively. After the heating, toluene was added to each sample and the heated meat was left at 25° for 72 hours. The amount of amino acid nitrogen produced in 10*g* of each heated sample was estimated. The amount of reducing sugar in the sample was also estimated.

Results obtained are shown in Table 36 and Fig. 22.

Table 36. Heat resistance of autolytic enzymes of crab meat

Heating temp. (°C)	Heating time (min.)	Amino-N (*mg*%)	Reducing sugar (*mg*%)
60	10	97	22.0
	15	85	10.0
70	10	80	10.2
	15	80	9.2
80	10	80	9.2
	15	76	9.0
90	5	76	9.0
	10	73	9.0
100	3	73	9.0
	10	73	9.0
Remarks (In initial sample)		73	9.0

for 14 days, but the top and bottom of the cans did not swell.

(1) Experimental method

Three cans (Nos. 1～3) which had been judged to be "flat sour" cans and one normal can (control), were opened in sterile condition, and a part of the content of each can was taken out with a sterilized pipette and inoculated into media by the usual method. Also, 150 *g* of the content of each sample can was subjected to paper chromatography. The rest of the content was used for the estimation of the amount of V.B.-N.

(i) *Detection of chemical components of soured smell*

The soured smell substance can be distilled by steam distillation. When S-benzyl-tiuronium chloride* was added to a part of the distillate, some crystals were formed out, and the distillate showed strong acid reaction. Therefore, this distillate would be considered to be acid substances such as volatile fatty acids.

The sample taken from the content of each can (3 soured cans and one normal can) were crushed in a mortar, respectively; hydroxamate was formed after Fink[17], from which the chemical components were detected by paper chromatography. The method of forming hydroxamate of fatty acids is shown in Scheme 1.

Scheme 1. Method of preparing hydroxamate of volatile acid

Raw crab 150 *g* + dist. water 200 *cc* + conc. H_2SO_4 15 *cc*
↓
Steam distillation
↓
200 *cc* Extract
↓
Extraction with ether (18 hours.)
↓
Evaporation of ether
↓
Brown liquid (1 *cc*)
↓
95 % ethanol 30 *cc* + conc. H_2SO_4 2 *cc*
↓
On oil bath, esterification (140°C)
↓
With sat. Na_2CO_3, neutralize of acid
↓
Heat about 70°C. (With reflux tube)
↓
Volatile acid ethylester
| Hydroxylamine hydrochloride 1.2 *g* + 7.5 *cc* alcohol + KOH 1.4 *g*
| ↓
| ←Filtrate ← KCl was removed
↓
Potassium hydroxamate
↓
Condensation
↓
Sample material of paper chromatography

* J.D. Leavy (1936) *J.A.C.S.* 58, 1004.

The hydroxamate obtained was used for paper partition chromatography, use being made of filter paper No. 50 (20×20 *cm*) of Toyo Filter Paper Co.

The sample was developed with water saturated n-butanol for 6 hours, and as spray reagent alcoholic solution of 2 % ferric chloride was used.

Before the detection of spots, the position of spots which appeared by application of pure formic acid, acetic acid, propionic acid, butylic acid and isovaleric acid was determined.

(ii) *Isolation of bacteria*

As "flat sour" cans are considered generally cuased by thermophiles, the contents of sample cans were subjected to bacteriological study. The media used were (1) liver-broth, (2) crab meat-broth (infusion of crab meat with 10 times volume of water, 100 *cc*, pepton 5 *g*, beef extract 5 *g*, NaCl 2 *g*; pH 7.2), (3) crab meat infusion agar, (4) glucose-agar, (5) Dyer & Snow's agar[16] (pepton 2 *g*, beef extract 2 *g*, glucose 1 *g*, NaCl 5 *g*, yeast extract 2 *g*, dipotassium phosphate 1 *g*, agar 1.5 *g* ,water 1000 *cc*; pH 7.2).

The inoculated media were incubated anaerobically (Burri's method, Manteufel method) at 37° and 50°C.

(2) Experimental results

From cultivated media, no bacterium was grown. Therefore, the cause of the soured decomposition was considered not to be bacterial origin.

The chromatograms obtained from each sample are shown in Fig. 23.

The amount of V.B.-N. was 32.4 *mg*% for No. 1 can, 31.7 *mg*% for No. 2 can, 35.2 *mg*% for No. 3 can and 26.9 *mg*% for the normal can.

As seen in Fig. 23, spots corresponding to formic acid, acetic acid, and propionic acid were observed for all samples of "flat sour" cans. In No. 2 can, the map which corresponded to butylic acid was observed, but not distinctly.

In normal can (control,) lower molecular acid such as formic acid was not detected, but propionic acid was detected. Besides this propionic acid, a spot was observed near 0.20 of R_f, but this spot was identified.

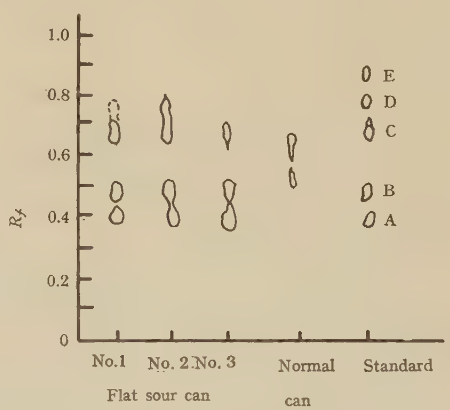


Fig. 23. Chromatograms obtained from the crab meat of "flat sour" cans and a normal can
A: Formic acid (0.40), B: Acetic acid (0.51), C: Propionic acid (0.70), D: Butylic acid (0.79), E: Isovaleric acid (0.84)

This spot may be considered to be some substance which was present in the original sample, or which was formed during the formation of hydroxamate.

The fact that spots revealed by the sample from the normal canned crab (control) were

less distinct than those from the "flat sour" cans, may be due to the presence of less amount of volatile acids in the normal cans.

From the results obtained, it seems that there were more kinds of volatile acids including lower molecular acids, and more amount of the volatile acid in the "flat sour" cans than in the normal can (control). That is to say, those volatile acids give the sour or stimulative taste of the canned material. This distribution of lower molecular fatty acids in the natural substances is limited except the acids having particular physiological functions. Therefore, those lower molecular acids are considered to be originated from the decomposition of amino acid of the protein of crab meat or from the lowering action of higher molecular fatty acids. The mechanisms of the decomposition or the lowering of amino acid of the protein of crab meat have been studied. The fact that the lower molecular volatile acids were found in the crab meat means that the final stage of decomposition has been reached. That is to say, the presence of lower molecular acids is considered to be created from amino acids or higher molecular fatty acids which are present in crab meat.

2. Relation between the degrees of freshness and the production of volatile acids

In the previous article, the author has presumed that the sour or stimulative tastes of the flat sour cans may be due to the presence of lower molecular volatile acids in the meat.

In order to know the reason why those substances were found without having been caused by bacterial decomposition in the canned food, study was made of the change of paper chromatograms of the chemical compounds created from the crab meat which had various degrees of freshness.

(1) Experimental method

The carapaces of *Paralithodes camtschatica* which were caught off Nemuro of Hokkaido were removed from the bodies after the landing. The raw crab meat of legs and shoulder was cut off from the crust.

The raw crab meat was broguht to the laboratory in a thermo's container with crushed ice. The raw crab meat was used as soon as possible after it reached the laboratory. The lapsed time was not over 30 hours after the capture. The same quantities of leg and shoulder meats were crushed and homogeneously mixed. An adequate amount of the homogenized meat was used for the determination by the same paper chromatography as described in the previous article. The spots revealed were determined, and compared with those noted in the previous article.

Raw crab meat having various degrees of freshness was made by leaving the raw meat in an ice box (5°C) for various lengths of time. The estimated or observed items were the same as those described in the previous article.

(2) Experimental results

The degree of freshness of raw crab meats which were employed for the estimation are

Table. 37. The amounts of V.B.-N and qualities in raw crab meat of various degrees of freshness

Leaving time (hrs.) (at 5°C)	V. B. -N (*mg*%)	Organoleptic inspection
0	8.6	Fresh, Sweet flavour
24	18.7	Less texture
48	31.1	Off flavour, Slight stimulous odour
72	50.5	Putrefactive odour

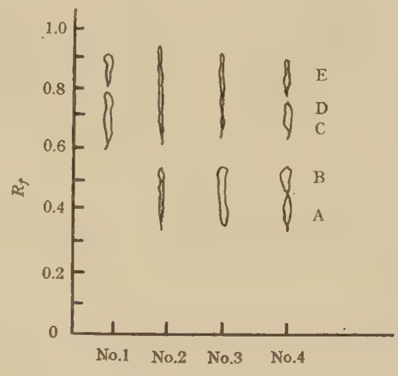


Fig. 24. Volatile acids of crab meat of various degrees of freshness
A : Formic acid (0.40), B : Acetic acid (0.51), C : Propionic acid (0.70), D : Butylic acid (0.79), E : Isovaleric acid (0.84)
No. 1……Fresh raw crab (V.B.-N, 8.6 mg%)
No. 2……Pretty unfresh raw crab (V.B.-N, 18.7 mg%)
No. 3……Unfresh raw crab (V.B.-N, 31.1 mg%)
No. 4……Very unfresh raw crab (V.B.-N, 50.5 mg%)

shown in Table 37.

The spots of volatile acids which were detected by paper chromatography are shown in Fig. 24.

As seen in Fig. 24, the spots obtained from fresh raw crab meat (V.B.-N, 8.6 *mg*%) were considered to show the presence of propionic, butylic and isovaleric acids. But slight appearance of the spots made difficult the identification of each substance. The spots obtained from pretty unfresh raw crab meat (V.B.-N 18.7 *mg*%) were identified to be acetic acid, and formic acid in addition to the isovaleric, butylic and propionic acids which were identified from fresh raw crab meat.

The spots obtained from unfresh raw crab meat (V.B.-N, 31.1 *mg*%) were identified to be the same kinds as those from pretty unfresh raw crab meat. In this case, the spots obtained were more clear than those obtained from pretty unfresh raw crab meat. The spots obtained from very unfresh raw crab meat (V.B.-N, 50.5 *mg*%) were identified to be butylic, propionic, acetic and formic acids. In this case, the spot to be identified for valeric acid was very much slighter than that observed from the pretty unfresh or unfresh raw crab meats.

The relation between the various degrees of freshness and the composition of volatile acids created in raw fish meat has been studied by many investigators[18,19,20,21,22]. According to Asakawa[22], the composition of volatile acids created was more or less

different by the kinds of fish,even though the degree of the freshness was almost the same. Therefore, it seems that no proportional relationship exsists between the composition of volatile acids and the degree of freshness of fish meat. However, in the case of crab meat the number of kinds and the amounts of volatile acids increased with the falling of the freshness. That is to say, with the falling of the freshness, volatile acids were found extending to the higher molecular fatty acids, if formic acid is left out of consideration. But that can not be decidedly declared on the basis of these experimental results only. It is rather considered that with the decompsition of raw crab meat, after the higher molecular compounds decomposed to lower molecular compounds or oppositely, composition of higher molecular compounds from the lower molecular compounds had taken place, the wider range of kinds of volatile acids was created.

In the present author's result, propionic acid was found even in fresh raw crab meat, but according to Asakawa, this acid was detected in the decomposed raw fish meat. No exact comparison can be made between the present author's result and Asakawa's result. In this case the factor of microorganisms as concerned with the process of the decomposition of the meat should not be omitted from this discussion. From this consideration, with the variation of the mode of the decomposition, the kinds of produced chemical components became greater.

Comparing the variation of the kind of volatile acids in the raw crab meat having various degrees of freshness with the kinds of volatile acids in flat soured cans of crab meat, one finds that the kinds of volatile acids in the raw fresh crab meat in this experiment were the same as those in the normal cans (control can for soured cans). Considering from the results that in the flat sour cans, formic, acetic and propionic acids were always found and butylic acid was sometimes found in some "flat sour" cans, with the fact that in the unfresh raw crab meat, the same kinds of volatile acids were found, the phenomenon of soured taste in the cans is presumed to be caused by the falling of the freshness of raw crab meat. But it is not safe to conclude that the volatile acids formed in "flat soured" cans are created from the raw crab meat of fallen freshness, because during the processing of canned crab, the raw crab meat is created by the boiling and retorting procedures, therefore changes of chemical components in the meat are considered to have taken place.

3. Relation between volatile acids in canned crab meat and the freshness of the boiled crab meat before the canning

It was ascertained that the amount of V.B.-N produced in the canned crab varies by the freshness of the raw crab meat. It is presumed that the amount or kind of volatile acids also in the canned crab varies by the freshness of the raw meat.

Hitherto, the relation between the volatile acids and the freshness of raw fish meat has been studied by many investigators[20,22]. The present author has also studied the variations in amount and kind of volatile acids in crab meat in connection with the freshness, and in the previous article has expressed the opinion that the soured taste in the "flat sour"

cans may be due to the falling of freshness of the crab meat before processing. Therefore the story of the various factors concerned during the processing of canned crab must be discussed.

In this article, when the boiled crab meat having various degrees of freshness was processed after the meat had been left in an ice box or at room temperature, the amount and the kind of formed volatile acids were studied.

(1) Experimental method

Raw fresh *Paralithodes camtschatica* meat taken from the bodies was boiled with the usual procedures. The boiled meat was left in an ice box or at room temperature, and thus material having various degrees of freshness was obtained. Those boiled meats of various degrees of freshness were processed into canned crab at various temperatures (2～6 pound pressure) for various times (60, 80 minutes) and the finished products were used for the estimation of volatile acids.

(2) Experimental results

The experimental results of analysis of above samples for the amount of V.B.-N and by organoleptic test are shown in Table 38.

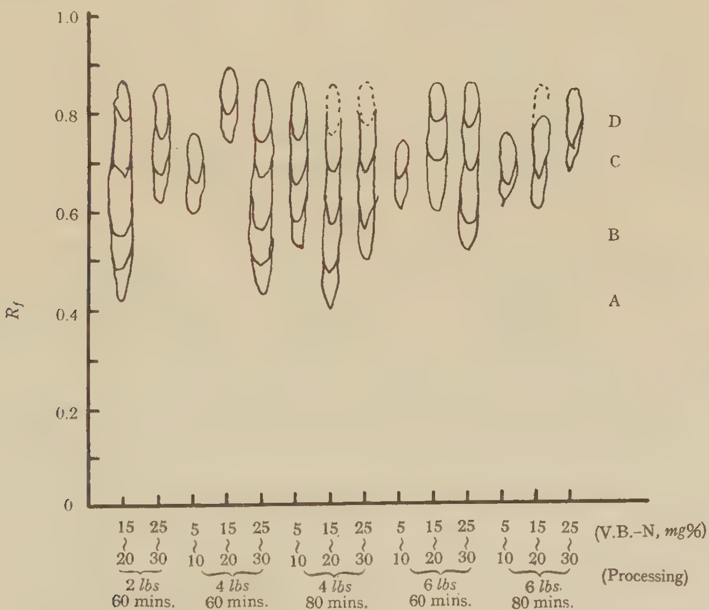


Fig. 25. Volatile acids of canned crab prepared from boiled meat of various degrees of freshness
A : Formic acid (0.40), B : Acetic acid (0.51), C : Propionic acid (0.70), D : Butylic acid (0.79)

Table 38. Opening inspection of canned crab prepared from boiled crab meat of various degrees of freshness

V. B.-N (Raw material) (*mg*%)	Processing time (mins.)	Estimating items \ Processing press. (*lbs*)	0	2	4	6
6～15	60	V. B.-N (Canned crab) (*mg*%)	7.8	11.1	13.8	15.8
		pH			6.2	6.0
		Aerobic bacteria	++	+	+	+−
		Anaerobic 〃	++	+	+	−
		Organoleptic inspection		Acid taste	Normal	Normal
	80	V. B.-N (Canned crab) (*mg*%)	7.8	13.7	21.5	20.5
		pH			6.2	6.2
		Aerobic bacteria	+	+	+−	−
		Anaerobic 〃	++	+	+	−
		Organoleptic inspection			Acid taste	Normal
15～20	60	V. B.-N (Canned crab) (*mg*%)	15.5	16.3	23.3	31.1
		pH		6.4	6.2	6.0
		Aerobic bacteria	+	+	+−	−
		Anaerobic 〃	+	+	+	−
		Organoleptic inspection	Acid taste	Acid taste	Acid bitter taste	Normal
	80	V. B.-N (Canned crab) (*mg*%)	18.7	25.0	25.4	33.4
		pH			6.0	6.2
		Aerobic bacteria	+	+	+−	−
		Anaerobic 〃	+	+	+−	−
		Organoleptic inspection	Acid taste, Stimulate odour	Acid taste	Acid taste	Normal
25～30	60	V. B.-N (Canned crab) (*mg*%)	24.7	26.1	31.0	38.9
		pH			6.2	6.6
		Aerobic bacteria	+	+	+	−
		Anaerobic 〃	++	+	+	−
		Organoleptic inspection	Acid taste, Stimulate odour	Acid taste	Acid taste	Normal
	80	V. B.-N (Canned crab) (*mg*%)	20.5	30.7	33.9	38.1
		pH		6.4	6.4	6.4
		Aerobic bacteria	+	+	+	−
		Anaerobic 〃	+	+	+	−
		Organoleptic inspection	Acid taste, Stimulate odour	Acid taste	Acid taste	Normal

Chromatogram of the composition of volatile acids is shown in Fig. 25.

As seen in Fig. 25, it will be observed that with the elongation of the leaving time of the boiled meat, the amount and the kind of volatile acids increased.

As to the processing temperature and time, there are few differences of the amount and the kind of volatile acids in the canned crab meats which were processed for various

periods in the range of this experiment at the same temperature. But when the processing temperature was changed, though there was no remarkable difference between the amount and kind of volatile acids in the canned crab meats which were processed at 103.6°C (2 pound pressure) or at 106.9°C (4 pound pressure), the number of kinds of volatile acids decreased in the canned crab meat processed at 109.9°C (6 pound pressure).

This may be due to the reason that the bacteria attached to the boiled meat which was filled into cans survived the processing at 103.6°C (2 pound pressure) or perhaps survived 106.9°C (4 pound pressure) and then caused decomposition of the crab meat forming volatile acids.

On the other hand, in the crab meat processed at 109.9°C (6 pound pressure), in which the bacteria were destroyed and the bacterial decomposition was prevented, the number of the kinds of volatile acids was small. The composition of volatile acids produced in the boiled meat left for various storing times will be changed by the heating, and the degrees of the change will be varied by the different processing temperatures.

When the processing temperature was 109.9°C (6 pound pressure), the molecular composition of volatile acids produced will become larger than those at other processing temperatures, 103.6°C or 106.9°C.

Those two considerations as above described may be due to the thermotolerance of the isolated bacteria. From the canned crab samples prepared by the experimental method as above described, the bacteria were aerobically and anaerobically isolated, and were tested for their thermotolerance. According to the results obtained, the isolated bacteria were able to survive at 107°C (4 pound pressure) for 20 minutes, but they were destroyed at 110°C (6 pound pressure) for the same time. Therefore, the bacteria present in canned crab could survive 2 or 4 pound pressure of processing, but were destroyed at 6 pound processing pressure.

Therefore, the change of the kinds of volatile acids after the processing may be influenced by bacterial action in the finished product rather than by the heating.

In this experiment, the samples from which formic acid was found, was one of canned crab processed at 103.6°C (2 pound pressure) for 60 minutes from the boiled meat left for two nights, and was processed at 106.9°C (4 pound pressure) for 80 minutes from the same boiled meat, or in canned crab processed at 106.9°C (4 pound pressure) for 60 minutes from the boiled meat left for three nights. In those cans the contents were understerilized. Accordingly it may be said that the canned crab which was processed from boiled meat left for two or three nights is apt to be understerilized, and in those cans, formic acid is possibly produced during storage after processing. However, when the cans were completely sterilized, the source of the production of formic acid must be considered to be from elsewhere.

From canned crab which was processed at 109.9°C (6 pound pressure) for 60 minutes or 80 minutes, no bacterium was isolated. In those cans processed from fresh boiled meat, the same components of volatile acids such as acetic acid and propionic acid were found

amongst components found in normal canned crab.

Against expection, in the canned crab processed from the unfresh raw crab meat, formic acid was not found, but only acetic, propionic and butyric acids were found.

In the canned crab processed at 109.9°C (6 pound pressure) for 80 minutes, propionic and butyric acids were found, but acetic acid was not found. Such differences in canned crab are considered to be caused by bacterial metabolism which will be caused by the multiplication of bacteria in the boiled crab meat during the leaving, or caused by the secondary decomposition of volatile acids which were once produced throughout the heating at the processing.

At any rate, except the case that fresh crab meat is processed, when the boiled crab from fresh raw material is even left and then processed, deterioration of the canned crab is normally differing from that which was processed from raw unfresh crab meat.

4. Composition of volatile acids in canned crab processed from the material having various degrees of freshness

In the previous article report was made on the composition of volatile acids in crab cans which were processed from the boiled meat having various degrees of freshness by leaving the boiled meat for various storage period. According to the results obtained, no difference of the kinds of volatile acids was observed as resultant from the freshness of the boiled meat.

In the next experiment, the raw crab meat was left for various lengths of time before the boiling, so the freshness of the raw meat was varied.

After the boiling the raw crab meat of various degrees of freshness, the meat was processed into canned crab. The composition of volatile acids was detected by paper chromatography.

(1) Experimental method

After the removing of carapace, the raw crab meat was left for some period of storage. Those raw crab meat lots having various degrees of freshness were processed at 109.1°C (5.5 pound pressure) for 85 minutes after the boiling. The samples of canned crab were opened, and a part of the can content was used for bacteriological test ; the rest was used for detection of the composition of volatile acids by paper chromatography and for the estimation of the amount of V.B.-N.

(2) Experimental result

Results obtained are shown in Table 39 and Fig. 26.

As seen in Table 39, no bacterium was isolated from the sample cans. Therefore, the sample cans were considered to have been completely sterilized at 109.1°C (5.5 pound pressure) for 85 minutes. As seen also in Table 39 and Fig. 26, the kinds and the amounts of volatile acids increased with the falling of the freshness of the raw crab meat material. In canned crab meat processed from fresh raw crab, acetic and propionic acids were found.

Table 39. Opening inspection of canned crab prepared from raw crab meat of various degrees of freshness

V. B.-N (Raw crab) ($mg\%$) / Estimating items of canned crab	6～10	15～20	25～30	35～40
Vacuum (*inch*)	17.5	16.5	14.0	23.0
Odour	Sweet flavour	Sweet flavour	Sweet, slight unpleasant	Unpleasant
Color of meat	Bright red	Bright red	Bright red	Discolorate
Quality of meat	Medium	Medium	Medium, softening	Softening
pH	6.6	6.8	6.6	6.8
Amount of Volatile acid ($mg\%$)	16.5	67.4	76.2	93.5
V. B.-N (canned crab) ($mg\%$)	23.4	25.5	34.2	49.4
Taste	Sweet	Sweet	Sweet, slight bitter	Acid

The revealed spot corresponding to propionic acid was more remarkable than that acetic acid. In canned crab meat processed from pretty unfressh raw material (V.B.-N, 15～20 $mg\%$), propionic acid and butyric acid were found. In canned crab meat processed from unfresh raw crab meat (V.B.-N, 25～30 $mg\%$), the kinds of volatile acids increased; formic, acetic, propionic and butyric acids were found. But in this case, the revealed spots became more remarkable with the increase of the molecular weight of each component. That is to say, spots of formic acid and acetic acid were slightly remarkable. In canned crab meat processed from very unfresh raw meat (V.B.-N, 35～40 $mg\%$), the kinds of volatile acids were the same as those in canned crab meat processed from unfresh raw meat (V.N.-B, 25～30 $mg\%$). In this case, the revealed spots of higher or lower molecular acids were both remarkable.

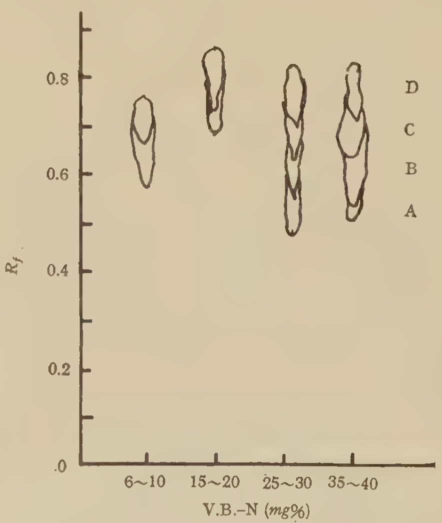


Fig. 26. Volatile acids of canned crab prepared from raw meat of various degrees of freshness

A : Formic acid (0.40), B : Acetic acid (0.51), C : Propionic acid (0.70), D : Butylic acid (0.79)

Comparing those results obtained from canned crab processed from raw crab meat having various degrees of freshness, with those results above obtained on material made from boiled meat having various degrees of freshness, when the raw crab meat was fresh (V.B.-N, 5～10 $mg\%$), acetic and propionic acids were found before the boiling, and the same acids

were found after the processing. In the case of pretty unfresh raw crab meat (V.B.-N, 15～20 *mg*%), formic acid, acetic acid, propionic acid, butyric acid and valeric acid were found before the boiling, and after the processing of the same boiled crab propionic acid and butyric acid were detected. In the case of unfresh raw crab meat (V.B.-N, 25～30 *mg*%), the same kinds of acids as above listed were found before the boiling, and also the same kinds of acids except valeric acid were detected after the processing. In the case of very unfresh raw crab meat, the same kinds of acids were detected both before boiling and after processing.

From those results, the kinds of acids in canned crab were ascertained to be the same as with these in raw material. That is to say, if there are many kinds of volatile acids in canned crab, the freshness of the raw material is considered unfresh. On the contrary, if there are few kinds of volatile acids in the canned crab, the freshness is considered to show fresh. The kinds of volatile acids in the canned crab, of which content was deteriorated were the same kinds as in the canned crab which was processed from unfresh raw material (V.B.-N, 25～30 *mg*%). The same kinds of volatile acids were found in canned crab processed from the boiled meat left for some period and of which the freshness fell. In raw crab meat, of which the freshness fell, valeric acid was already found before the boiling. But this acid can not be found in the canned crab processed after boiling from the same raw material. This may be considered due to the reason that some kinds of acids formed in raw meat flow out into juice at the boiling, for example valeric acid formed in unfresh raw meat flowed out into juice, therefore a small quantity of that acid perhaps remained in the canned crab. This matter is under further study in detail.

It was obviously shown that in that canned crab meat which was prepared from boiled meat left over two nights and processed at 103.6°C (2 pound pressure) for 60 minutes or at 106.9°C (4 pound pressure) for 80 minutes, and that which was processed at 106.9°C (4 pound pressure) for 60 minutes from the boiled meat left over three nights, formic acid was detected; the product tasted sourer than that in other cans. When raw unfresh crab meat in which formic acid had formed was packed into tin container, the meat tasted sour. In canned crab, of which the content deteriorated, formic acid and other acids were formed as above described. From those observations, sour taste may be due to the presence of formic acid. However, in a certain range of degree of freshness of crab meat, at which formic acid is detected, other volatile acids are also found remarkably, so the author wishes to interpret that the sour taste may be due to the increase in the total amount of all volatile acids due to the fallen freshness of the material.

From the fact that the series of volatile acids in the canned crab in which the quality of content is deteriorated, is almost similar to those in the fallen freshness of raw crab meat, the deterioration of canned crab is considered to be dependent upon the fallen freshness of the material before the processing.

According to the results obtained by experiments and observations, the series of volatile acids produced in raw crab meat except valeric acid, appear to go with the meat

into the container, but that is not yet quite clear. To the author's opinion, some quantities of volatile acids once produced in crab meat by the boiling, dissolve into boiling water, and the amount of such acids in the crab meat decreases, then the amount of volatile acisd in the crab meat after the processing again increases owing to chemical decomposition of the components of crab meat. Those considerations will be ascertained in future studies.

As the volatile acids responsible for the sour taste of crab meat were presumed to originate from some amino acid in crab meat protein, in order to ascertain the amino acid precursor the author has tried to detect the composition of amino acids in the meat protein of crab.

In two dimensional paper chromatography the HCl-hydrolyzed products were employed. According to the results obtained, aspartic acid and glutamic acid, alanine, valine, phenylalanine, leucine, serine and threonine were found as monoamino acids, while cystine, arginine and lysine were found as diamino acids.

It is known that those amino acids are decomposed anaerobically to the saturated fatty acids. For example, aspartic acid detected in this experiment changes to propionic acid *via* malic acid and succinic acid as seen in the following chemical formula[23].

$$\underset{\text{Aspartic acid}}{COOHCHNH_2CH_2COOH} \rightarrow \underset{\text{Malic acid}}{COOHCHOHCH_2COOH} \rightarrow \underset{\text{Succinic acid}}{COOHCH_2CH_2COOH} \rightarrow \underset{\text{Propionic acid}}{CH_3CH_2COOH}$$

It is easily presumed that aspartic acid which is abunduntly present in raw crab meat changes to propionic acid with the falling of freshness of the crab meat.

Beatty *et al*[24] have studied the glycolysis of fish and shell fish meats, and interpreted the process of the the production of acetic acid from lactic acid. In crab meat, glycogen changes to lactic acid during rigor mortis, the produced lactic acid will decompose reductively in the presence of trimethylamine oxide, to form acetic acid, trimethylamine and CO_2 by the following chemical formula.

$$\underset{\text{Lactic acid}}{CH_3CHOHCOOH} + \underset{\text{Trimethyl-amine oxide}}{2(CH_3)_3NO} + H_2O \rightarrow \underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Trimethyl-amine}}{2(CH_3)_3N} + 2H_2O + CO_2$$

Therefore, the precursors of sour tasting chemical components are considered to be present in the raw crab meat.

5. The influences of boiling procedure upon the composition of volatile acids in crab meat

As described in the previous article, in the raw pretty unfresh crab meat (V.B.-N, 15～20 *mg*%), formic, acetic, propionic, butyric and valeric acids were found before the boiling, but only propionic acid and butyric acid were found after the processing. In the raw unfresh crab meat (V.B.-N, 25～30 *mg*%), formic, acetic, propionic, butyric and valeric acids were found before the boiling, but valeric acid was not found after the processing. On the basis of those results, the author has presumed that volatile acids in raw crab meat dissolve out into the boiling water, though the amount is different for each acid, then the

amount decreases in the meat during the boiling procedure, at which large quantities of some volatile acids in crab were removed into boiling water, and that the remaining portions are retained. The author has also considered that volatile acid may increase again in crab meat on account of chemical change of meat tissue during the processing after the sealing of the cover.

Here, an attempt is made to clear the influences of the heating on the series of volatile acids.

(1) Experimental method

In practical procedures in crab canneries, crab carcasses were boiled in 3~4 *mg*% NaCl solution or in sea water. Those waters are changed after being used for boiling 4 or 5 lots of carcasses. In this experiment, raw crab carcasses having various degrees of freshness were boiled in 3% NaCl solution. A part of the saline solution was taken after 5 times of boiling. The series of volatile acids in raw crab meat were detected by paper chromatography as above described. Employing the crab meat, after the boiling process, the kinds of volatile acids in the boiling water were detected by adding ethyl ether to the water and agitating, leaving at 20°C for 18 hours thereby causing of the volatile acids into the ether layer.

(2) Experimental results

Results obtained are shown in Figs. 27~29.

As seen in Figs. 27~29, acetic acid and propionic acid were always found in raw crab meat. This result agrees with the results obtained as described in the previous article. If the pretty unfresh raw crab meat (V.B.-N, 15~20 *mg*%) was boiled, the series of volatile acids from formic acid to valeric acid were formed in the first time boiling water, but the spots of formic acid and valeric acid in chromatography were more slight than those of other acids. With the falling of freshness of crab meat, the spots became clear. That is to say, with the falling of freshness, larger amounts of volatile acids dissolved out into the boiling water.

In respect to the number of times of boiling, if the crab carcasses having the same degrees of freshness were boiled at different number of times, the kinds of volatile acids were almost the same without relation to the number of times of the boiling. For example, if the very unfresh raw carcasses were boiled, the kinds of volatile acids in the boiling water were almost the same as in cases of one time boiling or of five times boiling.

If the raw pretty unfresh crab carcasses (V.B.-N, 15~20 mg%) were boiled, the spots revealed of all the kinds of volatile acids in the boiling water after the first boiling were more clear than those after the fifth boiling. On the contrary, if the raw unfresh crab carcasses (V.B.-N, 25~30 *mg*%) were boiled, the spots after first boiling were less clear than those after the fifth boiling. With the increase in number of times of boiling, the number of kinds of volatile acids dissolved out into the boiling water is considered to increase correspondingly. Therefore, the results obtained from the raw unfresh crab carcasses meat can be considered

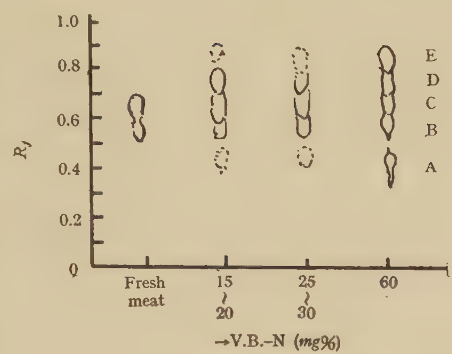


Fig. 27.

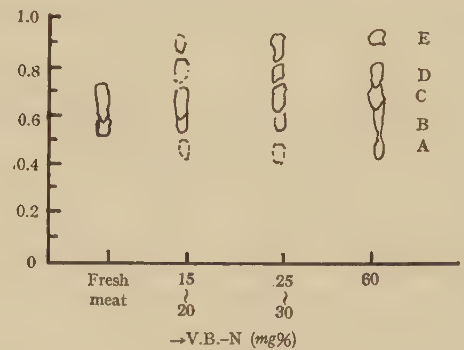


Fig. 28.

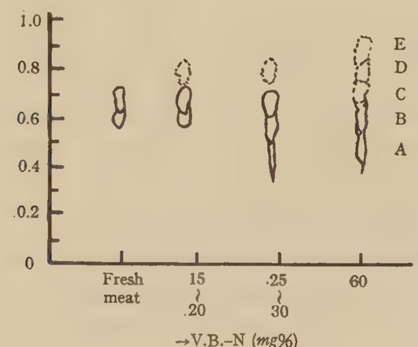


Fig. 29.

Fig. 27. Volatile acids in boiling water after one time of boiling of raw crab of various degrees of freshness

A: Formic acid (0.40), B: Acetic acid (0.51), C: Propionic acid (0.70), D: Butylic acid (0.79), E: Isovaleric acid (0.84)

Fig. 28. Volatile acids in boiling water after five boilings of raw crab of various degrees of freshness

A: Formic acid (0.40), B: Acetic acid (0.51), C: Propionic acid (0.70), D: Butylic acid (0.79), E: Isovaleric acid (0.84)

Fig. 29. Volatile acids in boiled crab meat after boilings of raw material of various degrees of freshness

A: Formic acid (0.40), B: Acetic acid (0.51), C: Propionic acid (0.70), D: Butylic acid (0.79), E: Isovaleric acid (0.84)

trustworthy. But the reason why the results obtained from the raw pretty unfresh carcasses are contrary is not yet clear. At any rate, it is considered that the boiling water can be used in the canneries for five times of boiling without changing.

As described in the previous article, in the fresh raw crab meat after the boiling, acetic acid and propionic acid were found. These sorts of volatile acids were the same as those in the first or in the fifth boiling water in which the raw carcasses were changed. From these

observations, the kinds of volatile acids may remain the same in crab meat even after the boiling.

If the pretty raw unfresh crab carcasses (V.B.-N, 15～20 mg%) are boiled, all the kinds of volatile acid were found in the boiling water, but acetic acid, propionic acid and butyric acid were found in the boiled crab meat itself. Comparing those results with that described in the previous article, when the pretty unfresh raw crab carcass meat (V.B.-N, 15～20 mg%) was processed after boiling, acetic acid, propionic acid and butyric acid were found in the canned crab meat; the kinds of volatile acids were almost the same in the boiled crab meat before the processing.

Therefore, the assumption seems disproven which was made in the previous article that some kinds of volatile acids in the crab meat disappear once as a result of boiling, but the kinds increase secondarily on account of the change of the meat after the processing.

Results obtained as to the unfresh crab meat showed almost the same tendency as the results described as above.

It is insufficient to state from the above assumption that the kinds of volatile acids produced in the crab meat before the boiling were not found in the meat after the boiling. Therefore, the facts that the kinds of volatile acids in fresh crab meat are the same before and after boiling, and that those in the boiling water and the boiled crab meat are different, lead to the following conclusion: the amounts of various kinds of volatile acids are not equal, therefore when those acids flowed out into the boiling water, the larger part of each acid which is present in less amount flowed out into the bioling water, and less amount of volatile acid which is present in larger amount will be left in the boiled crab meat, even if the larger part of those acids flowed out into the boiling water. Under this consideration, almost the total amount of formic acid or valeric acid which are produced in less quantity in comparatively fresh raw crab meat, will flow out in the boiling water, even if those acids are produced in the crab meat, therefore those acids will not remain to be detected in the crab meat. On the other hand, in the unfresh raw crab meat, formic acid, butyric acid and valeric acid will be produced in larger amount, so some part of the amounts of those acids will flow out, and another considerable part will remain in the crab meat.

From those facts, in the case when the pretty unfresh raw crab meat is boiled, if the boiling time and washing time are prolonged, the larger part of the amount of volatile acids will be flowed out. But with the increase of boiling and washing times, larger amounts of meat extractive will also be flowed out, so that taste will become inferior.

As clear in the previous results, when the raw crab meat is pretty unfresh or unfresh, many kinds of volatile acids remain in the crab meat even after the boiling. Those remained volatile acids will transfer into the can with crab meat and taste sour. This is considered to be a cause of "flat sour" of canned crab.

From the discussions offered above, the following conclusions are reached.

(1) Sour taste of the canned crab which is called "flat sour" has relation with volatile

acids. (2) With the falling of the freshness of raw crab meat, the amounts of volatile acids increase. (3) By the boiling of raw crab meat, a part of the amount of volatile acids produced in the raw crab meat is caused to flow out into the boiling water and if the produced amount in crab meat is comparatively large, the acids remain in the crab meat. (4) The kinds of volatile acid in the boiled meat are unchanged even after the processing. (5) The presence of formic acid in the canned crab is considered to take part in causing the sour taste. (6) When the raw crab meat is unfresh (V.B.N, 25~30 mg%) formic acid is found in the boiled crab met. (7) Therefore, in order to prevent "flat sour" of which the cause is without relation to bacteria, the fresh raw crab must be used.

SUMMARY

In this paper, the author has studied the troubles and technical problems attendant upon the processing of canned crab (King crab : *Paralithodes camtschatica*) which have been called to his attention.

In Article I, the decomposability of raw king crab meat was discussed from the view-point of the histological characteristics.

Then, the author ascertained that when the amount of volatile basic nitrogen (V.B.-N) in the raw crab meat rises over about 20 *mg*%, the meat becomes unfit for canning. Therefore in Article II, studies were tried on the relationship between the storing temperature and maximum storing time for which the crab meat can be stored and still remain suitable as raw material for canning. From the results obtained, a scale was prepared by which one can learn the maximum storing time at various temperatures of the raw crab meat from the standpoint of rational processing. But, in practice, it is rather necessary to know also the limit of freshness of the boiled crab meat and the maximum storing time before the limit is reached.

For the purpose, the author in Article III has reported on the variation of the freshness of boiled crab meat treated by the "high-" (at 100°C, 15 minutes) or "low temperature boiling method" (at 60°~65,° 10~15 mins), and the limit of the freshness of the boiled crab meat was determined from the view point of suitability as material for canning.

From the results of study, it is concluded that when the freshness of the raw crab meat is below 10 *mg*% of V.B.-N, the limit of the freshness of the boiled meat should be 15 *mg*%, and when the freshness of the raw meat is 10~20 *mg*%, the limit of the freshness of the boiled meat should be 10~13 *mg*%. When the freshness of the raw crab meat is above 20 *mg*%, even if the raw meat is boiled and processed for canning without delay, good quality of canned crab is not obtainable. At this points, the author prepared a scale showing the relation between the storing temperature of the boiled crab meat and maximum storing time for the canning, which it is also advisable to use in the rational processing.

Further, the determination of the processing time for canned foods according to the freshness of the raw or boiled material is very important. In Article IV, it is presented a

discussion on calculation of processing time of canned crab meat having different degrees of freshness; a consolidated diagram is prepared which shows the relation among the storage temperature of the raw or boiled crab meat, boiling methods, falling of freshness and the processing temperature-time of canned crab. The diagram may be convenient for the technologists employed in crab canneries.

In Article V, some problems were discussed concerned with canned crab processed by subjecting to the "low temperature boiling method" which has been invented by Kosakabe[2].

Especially, the author has studied the action of autolytic enzymes after the "low temperature boiling method". From the results, it was observed that the autolytic action of enzymes in the crab meat was maximum at 50°C, and was destroyed by above 90°C boiling.

At last, in Article VI, studies were described of "flat-sour"-like canned crab, of which the cause was ascertained to be not thermophilic bacteria, but to be due to the production of soured chemical substances by the falling of the freshness. The soured substances were identified as volatile acids, especially the presence of formic acid in the canned crab was considered to take a part in causing the sour taste. And, when the raw crab meat is pretty unfresh or unfresh, many kinds of volatile acids remain in the crab meat even after the boiling. Those volatile acids which are left will be transferred into the can with crab meat and taste sour. This is considered to be a cause of the "flat-sour" of this canned crab.

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